PB86-239837

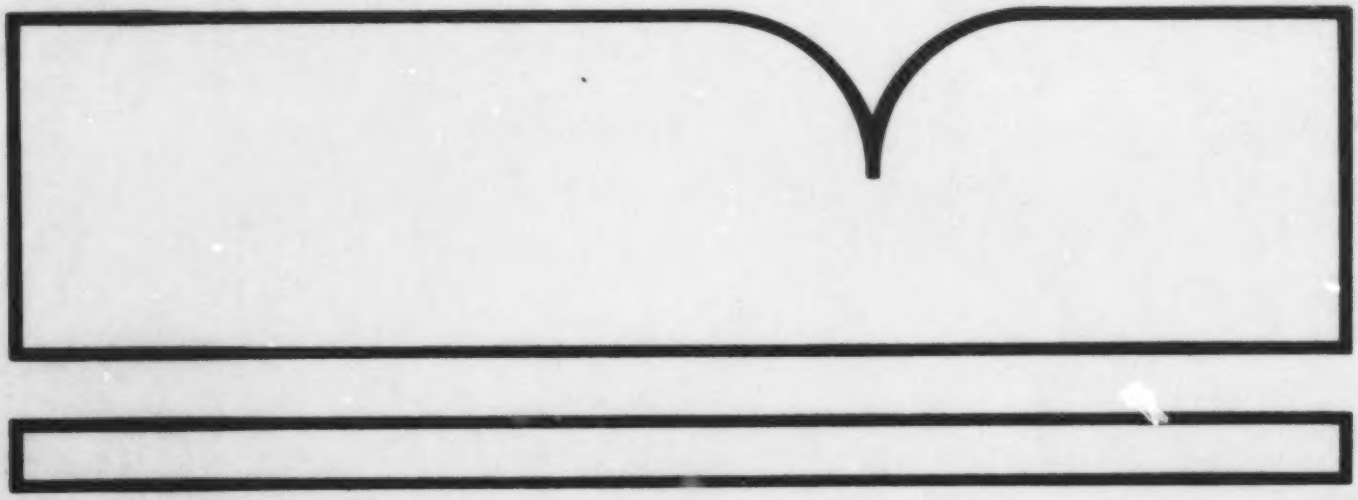
Mutagenic Activities of Wood Smoke Photooxidation Products

Northrop Services, Inc.
Research Triangle Park, NC

Prepared for

Environmental Protection Agency
Research Triangle Park, NC

Aug 86



U.S. Department of Commerce
National Technical Information Service
NTIS

PB86-239837

EPA/600/3-86/049
August 1986

MUTAGENIC ACTIVITIES OF WOOD SMOKE PHOTOOXIDATION PRODUCTS

by

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/3-86/049	2.
3. RECIPIENT'S ACCESSION NO. PB86 239837/AS	
4. TITLE AND SUBTITLE MUTAGENIC ACTIVITIES OF WOOD SMOKE PHOTOOXIDATION PRODUCTS	5. REPORT DATE August 1986
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) T. E. Kleindienst, P. B. Shepson, and E. O. Edney	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Northrop Services, Inc. Environmental Sciences P. O. Box 12313 Research Triangle Park, North Carolina 27709	10. PROGRAM ELEMENT NO. C9TA1B/02-6111(FY-86)
	11. CONTRACT/GRANT NO. 68-02-4033
12. SPONSORING AGENCY NAME AND ADDRESS Atmospheric Sciences Research Laboratory-RTP, NC Office of Research and Development U. S. Environmental Protection Agency Research Triangle Park, North Carolina 27711	13. TYPE OF REPORT AND PERIOD COVERED Final 10/84 - 7/86
	14. SPONSORING AGENCY CODE EPA/600/09
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

Irradiations of diluted wood smoke were conducted to assess the nature of the changes which are likely to occur to such a complex mixture in the atmosphere. The photochemical simulations were conducted for two distinctive chemical conditions: (1) diluted wood smoke alone, and (2) diluted wood smoke with nitrogen oxides (NO_x) added to bring the hydrocarbon to NO_x ratio more in line with that observed in populated areas. In all simulations, changes in chemistry and mutagenic activity were measured for both the gas-phase and aerosol-bound species. When diluted wood smoke alone was irradiated, the reaction proceeded quickly, but the extent of reaction was severely limited by the lack of NO_x. Nevertheless, the mutagenic activity of the vapor phase materials increased measurably. For the experiments with added NO_x, the photooxidation process was more complete, and a comparison of the mutagenic potential of the aerosol-bound chemicals with the gas phase products was possible. When compared on a mass basis (revertants per microgram of organic material) the mutagenic potency of the aerosol-bound products (and starting materials) is roughly equivalent to the potency of the gas phase transformation products. Most of the organic mass is in the gas phase, however, and when the potencies are compared on a volume basis (revertants per cubic meter of air sampled), the gas phase transformation products are by far the most potent.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 55
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (9-73)

REVIEW NOTICE AND DISCLAIMER

The information in this document has been funded by the United States Environmental Protection Agency under Contract No. 68-02-4033 to Northrop Services, Inc. – Environmental Sciences. It has been subject to the the Agency's peer and administrative review, and it has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ABSTRACT

Dilute mixtures of wood combustion emissions (with and without additional NO_X) were irradiated in a 22.7-m^3 Teflon smog chamber. The effluent was tested for mutagenic activity by exposing *Salmonella typhimurium*, strains TA100 and TA98, to the filtered gas phase components. The particulate matter was tested using the plate incorporation procedure. Without added NO_X, irradiated dilute wood smoke showed a measurable increase in mutagenic activity for gas phase products only. Additional NO_X was added in other irradiations to enhance the formation of gas and particulate phase products. Although only lower and upper limits were obtainable, the gas phase products showed considerably more activity (1.1 to 8.2 revertants/μg) in TA100 exposures than did the particulate product extracts (0.3 revertants/μg). With TA98 the activities of both phases were comparable on a mass basis (gas phase: 0.2-1.5; particulate phase: 0.9 revertants/μg). Since the total quantity of gas phase components was greater than the particulate phase components, the mutagenic activity on a volume basis was greater for the gas phase.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

CNC - condensation nuclei counter

EAA - electrical aerosol analyzer

GC - gas chromatograph

HC - hydrocarbon

MS - mass spectrometer

PAH - polycyclic aromatic hydrocarbon

PAN - peroxyacetyl nitrate (i.e., $CH_3COO_2NO_2$)

THC - total hydrocarbon

UV - ultraviolet

SYMBOLS

ΔHC - (absolute) difference between initial and final hydrocarbon concentrations

dt - differential with respect to time

t_{eff} - effective exposure time

t_{ex} - (actual) exposure time

ACKNOWLEDGEMENT

We thank G.R. Namie and J.H. Pittman of Northrop Services, Inc. – Environmental Sciences for their assistance in conducting the experiments, and E. Perry of Environmental Health and Research Testing for her assistance in conducting the biotesting. We also thank R.M. Kamens of the Department of Environmental Engineering, University of North Carolina, Chapel Hill, for his helpful discussions.

SECTION 1

INTRODUCTION

Recently, there has been increased concern that human exposure to wood stove and fireplace emissions may provide a public health concern. In a study of the Denver "brown cloud," Wolff *et al.* (1) determined that approximately one third of the organic fine particulate matter (<2.5 μm) from Denver aerosol could be attributed to wood smoke emissions. Although a more recent field study (2) indicates a fraction closer to 10%, the value can be highly dependent on the location of the sampling site. Several other studies have recently been reported (3, 4, 5) in which particulate extracts from wood smoke have been tested for mutagenic activity using the *Salmonella*/mammalian microsome reversion assay (6). These studies have generally shown low direct-acting mutagenic activity with *Salmonella typhimurium* strain TA98, although Dasch (3) reports substantial increases with the addition of S9 metabolic activation.

Kamens *et al.* (7) have reported substantial increases in the direct-acting mutagenic activity (TA98) of wood smoke when it was allowed to react in the dark with NO_2 and O_3. The Kamens *et al.* study (as well as those in references 4 and 5) focused on the particulate matter only, with emphasis on the mutagenic activity of the polycyclic aromatic hydrocarbons (PAHs). More recently, Kamens *et al.* (8) have determined that the PAH fraction of the particulate extract accounts for only a small fraction of the total mutagenic activity in both the reacted and unreacted wood smoke. The majority of the mutagenic activity resided in the more polar fractions of the extracts.

Two potentially important aspects of the health impact of wood smoke have not yet been addressed fully: (1) what is the effect of photooxidation reactions on the mutagenic activity of wood smoke? and (2) how does the relative mutagenic activity of the gas phase reactants and products compared to that of the particulate phase?

We have previously undertaken a series of studies to examine the mutagenic activity of product mixtures formed in the NO_X photooxidations of several hydrocarbons (HCs). We have found that the gas phase photooxidation products from an ethylene (C_2H_4)/NO_X irradiation are nonmutagenic to *S. typhimurium* strains TA98 and TA100 except for a small contribution in TA100 due to formaldehyde (HCHO; reference 9). Simple gas phase HCs, however, such as propylene (C_3H_6) and toluene (C_7H_8), are converted to gas phase photooxidation products that exhibit strong mutagenic activity with strains TA98 (10) and TA100 (10,11). In each of these systems, a large fraction of the total mutagenic response could not be accounted for based on the mutagenic

activities of individual products. In another study (12), strains TA98 and TA100 were exposed to irradiated mixtures of ethylene/aniline/NO_X. In this case, the gas phase products showed no activity in either strain, but a sizable response was observed in TA98 from extracts of particulate matter formed during the irradiation.

Since there are significant amounts of toluene and propylene in the gas phase wood smoke emissions (4), it would seem likely that the gas phase photooxidation products of wood smoke would show some activity and are thus worthy of consideration. In this paper, we report the results of several experiments in which the total mutagenic response in TA98 and TA100 was measured for dilute mixtures of wood smoke irradiated in the presence and absence of added NO_X. The mutagenic activities of the reactants and products were determined using a modified version of the Ames test (7) for gas phase species and the standard plate incorporation test (6) for particulate phase species. The data are analyzed to allow a comparison to be made between the relative amounts of mutagens in the gas and particulate phases for both reacted and unreacted wood smoke mixtures.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

We demonstrate in this study that whereas the gas and particulate phase components of wood combustion show little direct-acting mutagenic activity, the mutagenic activities of the gas phase (as measured using TA100 and TA98) and particulate phase (with TA98) species increase significantly upon irradiation in the presence of NO_X. In addition, when measured in terms of revertants/μg, the gas phase products are ~3-30 times more mutagenic than the particulate phase products as measured with strain TA100, and the mutagenic activities are comparable for the two phases with TA98. When compared on a per cubic meter basis, the gas phase products are far more mutagenic than either the starting materials or the particulate products. In light of the great diversity and number of studies of the mutagenicity of complex air mixtures in which the particulate phase has been the focus of attention, this study clearly indicates that gas phase species should be considered as well and that they could be very important.

SECTION 3

EXPERIMENTAL PROCEDURES AND MATERIALS

A schematic diagram of the wood stove dilution system, reaction chamber, and exposure chamber (i.e., biochamber) system is presented in Figure 1. Wood combustion for these experiments was conducted using an Ashley Model 7150B wood stove. The wood used was a mixture of oak varieties obtained locally in the Raleigh-Durham, NC, area. No attempt was made to investigate the dependence of the mutagenic activity on wood types or burn conditions, and the subject has been treated elsewhere (4). The wood stove was initially operated under conditions of relatively fast burn rate (with vents open) for all experiments. As the burn proceeded, the rate of combustion became slower and thus the reactant mixture represented an integrated mixture of fast and slow burn emission components. To load the reaction chamber, a portion of the wood smoke from the chimney was continuously drawn through an 8-cm steel pipe to a dilution tunnel where it was diluted with ambient air. The unfiltered ambient air was drawn through the dilution tunnel using a 0-20 m^3/min turbine. The dilute wood smoke could then be added to the reaction chamber (at 30 L/min) using a Metal Bellows Corporation Model MB-151 metal bellows pump.

The reaction chamber is a 22.7-m^3 cylindrical vessel constructed of 0.13-mm Teflon that is sealed to aluminum end plates coated with fluorocarbon paint. The chamber is surrounded longitudinally with a combination of sun lamps and ultraviolet (UV) blacklights. The light intensity is measured continuously during the irradiation using a radiometer. In a previous experiment the radiometer reading had been correlated to an equivalent NO_2 photolysis rate (0.3 min^{-1} for these experiments). Additional details of the reaction chamber are presented elsewhere (10). For some of these experiments, additional NO was added to the chamber from a 1% mixture of NO in N_2 (MG Scientific) by metering this mixture through a mass flow controller and allowing it to mix with clean dilution air in the 150-L stainless-steel inlet manifold, as shown in Figure 1.

Four 190-L rectangular biochambers were employed for the exposure of *S. typhimurium* to the gas phase mixtures: a clean air biochamber, a reactants biochamber, an ambient air biochamber, and the reaction chamber effluent biochamber. The air masses were transferred to each of the biochambers through 9.5-mm Teflon tubing at a flow rate of 14 L/min maintained and measured with a needle valve and rotameter. Teflon-impregnated glass fiber filters (T60A20 Pallflex 13.34 cm) were used to remove particulate matter from the reactants, effluent, and ambient biochamber air masses, as shown in Figure 1. The particulate matter collected on these filters was then used for

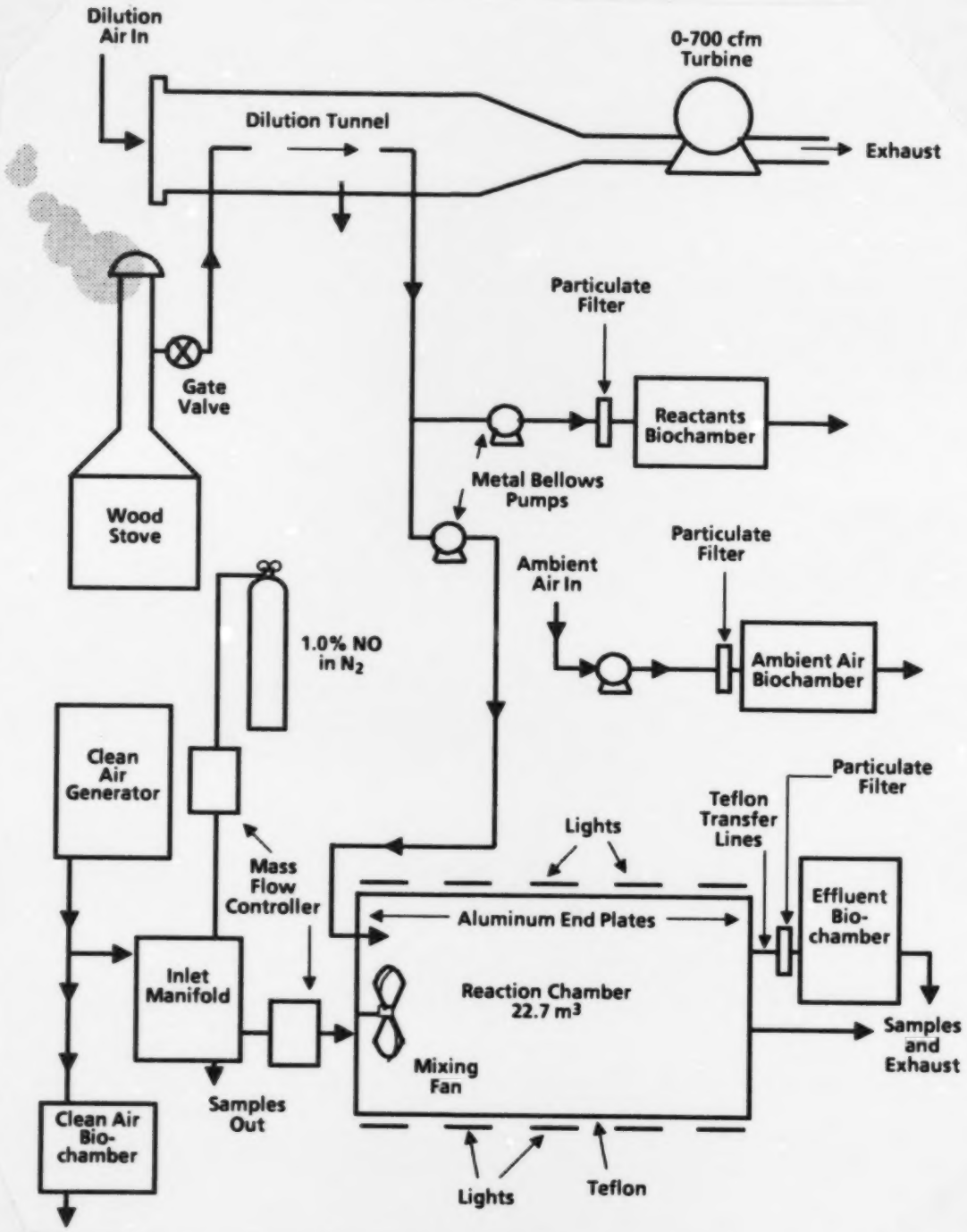

Figure 1. Experimental schematic of the wood stove, reaction chamber, and exposure apparatus.

bioassay of the particulate phase mutagens. Filter loadings from the reactant and effluent air streams ranged from 1 to 10 mg per filter depending on particle concentration and sampling time.

Sample collection for gas phase and particulate phase species was done at the end plate opposite the inlet manifold. The analytical methods employed for measurement of NO_X, O_3, peroxyacetyl nitrate (PAN), and the aldehydes were as previously described. Particulate matter concentrations were measured using a condensation nuclei counter (CNC; reference 10).

Methane, acetylene, ethylene, and ethane were measured with a Varian 1400 gas chromatograph (GC) using a 3.2 mm × 2 m stainless-steel column packed with 60/80 Carbosieve G, operated isothermally at 150°C. Chloromethane, propylene, propane, and 1-butene were measured with a Varian 1200 GC using a 6.4 mm × 2 m stainless-steel column packed with 80/100 Porapack QS, operated isothermally at 130°C. Isoprene, furan, and 2-methylfuran were measured with a Hewlett-Packard Model 5840A GC using a 2 mm × 2 m glass column packed with 0.1% SP-1000 on 80/100 Carbopack C, temperature programmed from 40°C to 200°C at 20°C/min. Sample injection for these three chromatographs was performed using heated Seizcor gas sampling valves with 5-mL sample loops. Calibrations were conducted by diluting samples of the pure compound with zero air in 100-L Teflon bags to concentrations in the low ppm range. Benzene, toluene, xylenes, benzaldehyde, styrene, and *m*-methylstyrene were measured by pumping 25-L samples through Pyrex tubes packed with Tenax GC. The Tenax-filled tubes were then thermally desorbed at 275°C with a Nutech 320 thermal desorption unit. The desorbed samples were analyzed with a Hewlett-Packard 5985 GC/MS containing a 2 mm × 2 m glass column packed with 10% SP-1000 on 80/100 Supelcoport. The column was temperature programmed from 50°C to 225°C at 20°C/min. Calibrations were performed by desorption of Tenax tubes containing samples of the pure compounds at the 1-nmole level. CO was measured using a Bendix Model 8501-5CA infrared CO analyzer. SO_2 was measured using a Meloy Labs Model SA285 sulfur analyzer. Total HC content was measured using a Beckman Model 400 HC analyzer. The HC analyzer was calibrated in units of parts per million of carbon (ppm of C) using pure samples of propane. Aerosol size distributions (0.01-1 μm) were measured with a Thermo Systems, Inc., Model 3030 electrical aerosol analyzer (EAA).

The particulate phase filter samples were Soxhlet extracted with 250 mL of pesticide-grade methylene chloride (Fisher Scientific) for 6 h. The extracts were then concentrated under a stream of prepurified N_2 (MG Scientific) and analyzed for PAH concentration using the GC/mass spectrometer (MS) by injection onto a 2 mm × 2 m glass column packed with 3% SP-2250 on 80/100 Supelcoport. The column was programmed from 200°C to 300°C at 10°C/min. For the compounds measured, the GC/MS peak areas were measured relative to pyrene and quantified as pyrene. (The reported concentrations are, therefore, accurate only to within a factor of two. However, this degree of accuracy was deemed adequate for the purpose of this study.) The extracts were prepared

for bioassay by solvent exchanging a portion of the methylene chloride extract with 2-mL samples of dimethyl sulfoxide (Burdick and Jackson Laboratories, Inc.). Blank filters were also extracted and served as both chemical and bioassay controls.

To measure potassium (K) and iron (Fe) concentrations in the particulate phase (the K/Fe ratio has been used as a wood smoke tracer [1]), particulate phase samples were collected on Millipore type AA, 0.8-μm cellulose filters. These were then digested in acid-washed glassware with 2 M high purity nitric acid (HNO_3) (Baker Ultrex; Fe < 0.5 ng/g) at 100°C. The K and Fe concentrations were measured using an Instrument Laboratories Corp. atomic absorption analyzer, which was calibrated using certified Fe standard solutions (Fisher Scientific) and in-house standards for K.

The bacteria *S. typhimurium*, strains TA100 and TA98, are used as the biological assay for this study. (Strain TA100 mutates by a base-pair substitution mechanism, and TA98 by a frameshift mechanism.) The plates were prepared by adding 0.1 mL of the *S. typhimurium* culture to 3 mL of an agar overlay at 45°C, with or without 0.5 mL of S9 mix. This mixture was then poured onto ~45 mL of plate agar in a Pyrex glass petri plate. The *S. typhimurium* tester strains were provided by Dr. Bruce Ames (University of California, Berkeley, CA). Colony counting was done with an Artec 880 automatic colony counter using previously published guidelines (13). The test procedures used were those of Ames *et al.* (6), except for the following modifications: (1) glass petri dishes were used, (2) 45 mL of base agar per plate were used, (3) minimal histidine at the same final total concentration was placed in the bottom agar rather than the top agar, and (4) 3 mL of overlay agar with $\sim 1 \times 10^8$ bacteria were used. The rat liver homogenate (S9) fraction was from male Charles River CD-1 rats (Wilmington, MO) induced with Aroclor 1254 (6).

The particulate extract bioassays were performed using the standard plate incorporation test. Bioassay of the gas phase species was conducted by exposing uncovered test plates to the air masses that flow through the biochambers. Plate dosage occurs as gas phase species dissolve into the agar during the exposure, as described in detail in a previous publication (11).

The experiments were conducted by first loading the reaction chamber to a total HC level of ~18 ppmC. The residence time of the air mass in the reaction chamber was ~6 h during loading, and therefore the final air mass represents an integration of wood stove burn conditions over this time scale. Prior to irradiation in Experiments B and C, NO was slowly added to the chamber until the desired initial concentration of ~0.6 ppm NO_X was reached. Experiments with added NO were conducted both as a means of slowing the reaction (to facilitate analytical measurements) and of controlling the extent of HC conversion. Once the desired initial conditions were reached, the chamber lights were turned on, thus starting the photochemical reactions. Dilution air was added at ~10 L/min to compensate for an equivalent sampling rate during the irradiation. The irradiations

were conducted until the PAN concentration leveled off, indicating that all NO_2 in the system was removed. (This corresponded roughly to the O_3 maximum.) The lights were then turned off, and the final reaction chamber samples were acquired.

The exposure of the biological assay to the product mixture was then conducted by flushing the reaction chamber contents through the exposure chamber at a known flow rate. The reaction chamber species concentrations were, therefore, continuously being diluted at this rate. Because it was difficult to maintain a constant output from the wood stove, this "static" exposure technique was employed, rather than the dynamic exposure which has been used in previous work (10, 11). Three other biochambers employed as controls were used to measure the possible mutagenic activity of the clean air, ambient air, and initial (wood combustion) reactants. Once the biochambers were adequately flushed with the appropriate exposure air, the plate covers were removed, effectively starting the exposure. A flow of 14 L/min was maintained through each of the biochambers during the exposure. Groups of plates were covered at various times during the exposure (0-10 h) so that dose-response curves could be constructed. Each biochamber was loaded with 24 test plates and 6 survivor plates for each strain. The survivor plates were prepared by diluting the 3-mL culture so that the bacteria concentration was ~500 /plate. Additional histidine was added to the survivor plates so that all bacteria grew to form colonies in the absence of toxic effects. The survivor counts therefore reflect the degree to which the test plates may experience toxicity. Dilution air at 20 L/min was added to the reaction chamber during the exposure to account for an equivalent sampling rate for chemical analysis and the effluent feed for the biochamber. The air mass for the reactant biochamber was prepared by maintaining the gas and particulate reactants (in the dilution tunnel) at the same concentration as those in the reaction chamber at the start of the irradiation. This mixture flowed continuously from the dilution tunnel to the reactants biochamber during the course of the exposure. At the end of the exposure, the plates were then covered, removed from the biochambers, and incubated at 37°C under sterile conditions for 48 h, and the number of revertant colonies per plate was counted. The filters used to remove particulate matter from the biochamber air masses were removed and extracted for the bioassay.

The results for four irradiations are reported here. In Experiment A, the exposure was conducted with combustion NO_X only and an initial HC and NO_X level of 20 ppmC and 0.13 ppm, respectively. Experiments B and C (March 7 and March 28, respectively) were performed as duplicates of each other and conducted at initial HC and NO_X levels of ~17 ppmC and 0.6 ppm, respectively. Experiment D was also conducted with added NO_X. In Experiment D the exposures were performed with the biological assay containing S9 metabolic activation. Several additional static runs were performed, mainly for chemical analysis, for which particulate phase biological assay data were also obtained.

SECTION 4

RESULTS

WOOD SMOKE IRRADIATION WITH COMBUSTION NO_X ONLY

Figure 2 shows the reactant and product profiles for a number of major species present in the wood smoke, gas phase system. Since the only source of NO_X was from the wood combustion, the HC/NO_X ratio was extremely high (~100:1). The photooxidation proceeded very rapidly, but the extent of reaction was severely limited by the lack of NO_X. The light intensity corresponded to a photodissociation rate for NO_2 of approximately 0.3 min^{-1} during the 2-h irradiation. (A reasonable solar zenith angle under wintertime conditions of approximately 60° during midday would correspond to an atmospheric photolysis rate for NO_2 of about 0.38 min^{-1} (14). In general for these experiments the integrated radiation with respect to NO_2 photolysis is less than that expected for an average clear winter day.) As observed in Figure 2, NO_X-NO reached its maximum concentration in less than 5 min, and the O_3 maximum occurred within an hour.

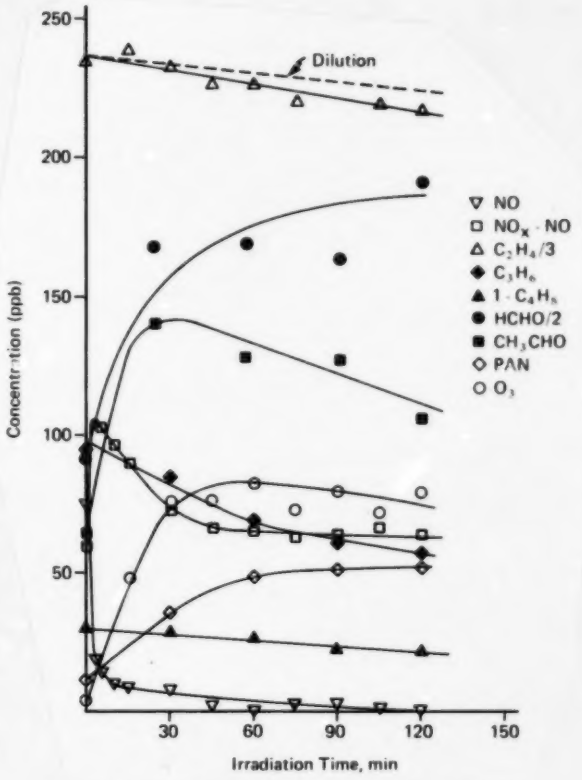

Figure 2. **Wood smoke static irradiation.** Selected reactant and product profiles. Experiment A, combustion NO_X only.

The four sets of columns in Table 1 (Experiments A, B, C, and D) give the concentrations of many of the gas phase reactants and of some of the products immediately before and after irradiation. In molar quantities, the major reactive HCs included alkenes (ethylene, propylene, and 1-butene), aromatics (benzene, toluene, xylenes), and oxygen atom heterocycles (furan, 2-furaldehyde, and 2-methylfuran). A large component of the gas phase emissions of the wood stove

was aldehydes, the two main species being HCHO and acetaldehyde (CH_3CHO). These two compounds existed as both reactants and products in this system. For example, the oxidation of propylene leads to the production of HCHO and CH_3CHO. The photooxidation of aromatics (toluene and xylenes) has been shown (15) to yield glyoxal ($[CHO]_2$), methylglyoxal (CH_3COCHO), and biacetyl ($[CH_3CO]_2$), all of which were also present in our experiments as reactants.

TABLE 1. INDIVIDUAL GAS PHASE INORGANIC AND HYDROCARBON CONCENTRATIONS,[a] ppb

Species	A (April 18)		B (March 7)		C (March 28)		D (April 10)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Nitric oxide	75	0	454	0	461	0	449	0
NO_X	135	64	657	252	576	259	553	243
Ozone	0	79	0	467	0	696	2	420
Carbon monoxide, ppm	33.5	32.0	38.0	33.4	38.7	35.5	39.8	35.2
Methane	–[b]	–	5060	4500	4480	3920	5000	4230
Ethane	–	–	146	135	–	–	–	–
Propane	–	–	9	9	–	–	–	–
Ethylene	702	652	537	313	847	439	728	468
Propylene	92	56	126	8	100	7	94	10
1-Butene	30	22	58	3	39	0	44	3
Isoprene	7	0	–	–	7	0	–	–
Acetylene	–	–	177	155	–	–	–	–
Benzene	68	62	62	50	102	68	66	45
Toluene	27	16	62	15	24	10	16	9
m- + *p*-Xylene	14	7	40	19	12	3	8	4
o-Xylene	5	3	13	4	2	0	2	1
Styrene	10	2	22	0	7	0	5	0.3
m-Methyl styrene[c]	5	–	9	0	3	0	2	0.4
Furan	49	21	71	0	59	0	46	0
2-Methylfuran	80	23	61	0	64	0	46	0
Formaldehyde	325[d]	381	269	365[e]	229	383	257	339
Acetaldehyde	140[d]	106	88	109[e]	57	75	73	82
Acetone	50[d]	41	–	–	–	–	44	22
Acrolein	35[d]	22	–	–	–	–	37	36
Benzaldehyde	–	–	15	12	–	–	–	–
2-Furaldehyde	48[d]	19	31	2[e]	22	6	30	3.3
Glyoxal	24[d]	16	41	45[e]	26	24	9.5	25.2
Methylglyoxal	–	–	41	36[e]	13	16	11.3	15.7
Biacetyl	22[d]	25	–	–	7	5	8.6	5.5
PAN	0	52	0	174	0	232	5	186
Chloromethane	–	–	18	18	–	–	–	–
HC[f], ppmC	20.6	19.1	16.4	13.2	17.2	15.0	15.7	13.2

[a] Other gas phase species identified: phenol, butadiene, methyl acetate, 2-methyl vinylacetylene, methyl vinyl ketone, methyl ethyl ketone, and benzofuran.
[b] Not measured.
[c] Tentatively identified based on mass spectrum.
[d] @ 0.4-h irradiation time.
[e] @ 1.5-h irradiation time.
[f] Corrected for dilution.

Physical and chemical characterization of the particulate phase was obtained both before and following irradiation. The number distribution of the aerosols was similar to that observed by other researchers (3, 7); that is, the vast majority of unreacted particulates were less than 1 μm in size, with the maximum of the number distribution occurring at approximately 0.1 μm. In Experiment A, the maximum in the number distribution shifted to 0.2 μm after irradiation. The number and the volume distributions of the particulate reactants and products as measured by the EAA are given in Appendix A, Figures A-1 and A-5, respectively. Although the absolute number of particles following irradiation was smaller than before irradiation (Table 2), the contribution to the volume (and thus the mass) of the particulate matter increases as r^3, giving the volume distribution for products shown in Figure A-5, with a peak at 0.4 μm. This increase in volume following irradiation has been observed in all our photooxidation experiments with wood smoke. It would seem likely that the increase in the total volume of particulate matter was the result of polar gas phase photooxidation products adsorbing onto the particulate matter already present. In the absence of irradiation, neither the number nor the volume distribution maximum of the particles changed significantly with time while they were in the chamber.

TABLE 2. PARTICULATE EXTRACT DATA, ng/mg PARTICULATE[a]

Species	A (April 18)		B (March 7)		C (March 28)		D (April 10)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Fluoranthene	647	47	721	70	611	203	772	28
Pyrene	602	57	672	50	560	218	866	36
Anthracene + Phenanthrene	256	50	457	100	132	89	303	34
Acenaphthene	454	37	371	30	103	≤ 100	173	≤ 100
Fluorene	261	33	214	20	224	≤ 100	116	≤ 100
Chrysene + Triphenylene	272	76	–	–	100	≤ 100	574	64
CNC ($\times 10^9$ particles/m^3)	18	15	33	28	13	10	36	26
EAA (nL/m^3)	137	232	286	591	72	265	133	280

[a] Other particulate-phase species identified: 4-hydroxy, 3,5-dimethoxybenzaldehyde; 4-hydroxy, 3-methylbenzoate; 4-hydroxy, 5-methoxy benzoic acid; benzo(a)pyrene.

Various particulate phase organic species were quantified from analysis of the filter extracts. The results for several PAHs that have been identified are given on a mass per extractable mass basis in Table 2. For each compound presented, significant degradation was observed as a result of the irradiation. Part of the apparent degradation occurred solely as a result of the increase in particulate mass (due to adsorption from the gas phase) as discussed previously. However, some reaction of the PAHs was apparent, although no photolysis or reaction products from these compounds were observed.

The dose-response curves for the bioassay results for the exposure of TA100 to the gas phase products of Experiment A are shown in Appendix B. (As implied by Figure 2, the exposure was conducted for the product distribution at an extent of reaction of 2 hours.) The mutagenic activities of clean air and ambient air are within the experimental error of the background spontaneous levels (188 ± 16 revertants per plate). Each dose for the reactants and effluent represents an average from eight plates randomly distributed within the exposure chambers.

For this experiment, the actual exposure times were 2.5, 5, and 10 h. However, since there was significant dilution during the course of the exposure (0.055 h^{-1}), the chamber concentrations were continuously reduced exponentially. The exposure times have been corrected to account for these continuously decreasing concentrations. The effective exposure time, t_{eff}, is given as

$$t_{eff} = \int_0^{t_{ex}} exp(-kt)\, dt \qquad (1)$$

where t_{ex} is the exposure time in real time and k is the dilution rate constant. The longest effective exposure time for an actual time of 10 h is 7.78 h. From the slope of the dose-response curve (Figure B-1), we observe a linear reversion rate with exposure time and calculate a slope of 36 revertants $plate^{-1}$ h^{-1}. The data for all gas phase exposures without metabolic activation are presented in Table 3.

TABLE 3. MEASURED MUTAGENIC ACTIVITIES OF THE GAS AND PARTICULATE PHASES OF IRRADIATED WOOD SMOKE[a]

	Gas (revertants/h)		Particulate (revertants/µg)	
	TA100	TA98	TA100	TA98
Combustion NO_X				
Reactants	−5.9 ± 2.4[b]	(-0.42 ± 0.86)[c]	0.29 ± 0.12	0.20 ± 0.09
Products	35.8 ± 5.5	3.37 ± 0.90	(0.10 ± 0.23)	0.26 ± 0.10
Added NO_X				
Reactants	(1.6 ± 2.7)	(-0.4 ± 1.2)	0.30 ± 0.10	0.22 ± 0.06
Products	174 ± 16	30.3 ± 3.7	0.27 ± 0.15	0.94 ± 0.30

[a] Values represent slopes from the dose-response curves. The uncertainty given is the 95% confidence interval.
[b] Survivor data indicate no toxicity. Slight negative response probably a reflection of the large relative uncertainty with a small response.
[c] Values in parentheses are zero within the uncertainty.

In Table 3 (and Figure B-2) we also present the results for the exposure of TA98 to the gas phase effluent for Experiment A. Again for this exposure, the clean air, ambient air, and reactant chambers' revertant levels per plate were within the experimental uncertainty of the spontaneous revertant level (41 ± 6 revertants/plate). The exposure times for the reactant and effluent were the

same as for TA100. For both TA100 and TA98, the survivor plates at each exposure time showed no sign of toxicity.

Table 3 also indicates the mutagenic activity (revertants/μg) of the particulate phase reactants and products for TA100 (Figure B-3) and TA98 (Figure B-4), respectively. A filter upstream of the ambient air exposure chamber was also extracted and tested. In no case did the ambient air extracts show a mutagenic activity above the spontaneous reversion level for either strain, although the total mass collected on the ambient air filters tended to be very small (< 100 μg). Each value in the table is based on four to six extract doses. For Experiment A (combustion NO_X only), the particulate extract data for TA100 show no increase in the mutagenic activity of the products over the reactants. For TA98, the products show a slight increase in activity but it is less than the experimental uncertainty (~0.1 revertants/μg).

WOOD SMOKE IRRADIATION WITH ADDED NO_X

Because the extent of reaction in Experiment A was limited, an accurate comparison of the gas to the particulate phase product mutagenicities was difficult to make. Experiments B and C were conducted with additional NO added to reduce the HC/NO_X ratio to approximately 25. Urban ratios usually range from 5 to 15 (16). The addition of NO served to convert HO_2 to OH during irradiation, and the oxidation of reactive HCs under the conditions with added NO tended to be more extensive. Product formation was significantly increased because OH was the primary oxidizing species in this system. (The NO_2 produced substantially increased O_3 formation.) This addition of NO_X effectively slowed the initial reaction rate.

The initial conditions for the experiments with added NO_X are given in Table 1 under the columns for Experiments B and C. The degree of reproducibility when adding wood smoke reactants to this system is indicated by comparing the initial conditions for these experiments. The time profiles of the major reactants and products for Experiment B are seen in Figure 3. The increased degree of oxidation of the major alkenes, ethylene, propylene, and 1-butene, is apparent. The formation of O_3 and PAN is extended in time, with the concentration maxima occurring past two hours. The formation of all major products is significantly greater than in the case without added NO_X. Chloromethane was measured in Expt. B because it is one of the few chlorinated compounds generated in wood combustion (17), and its potential as an atmospheric tracer has been recognized.

The particulate extract PAH data for these experiments are tabulated in the second and third sets of columns in Table 2. The upper limits appearing in the Experiment C data set reflect the relatively low quantity of particulate matter collected during the exposure (the maximum exposure time was 6 h for this experiment). For these compounds, the GC/MS detection limit was ~10 ng.

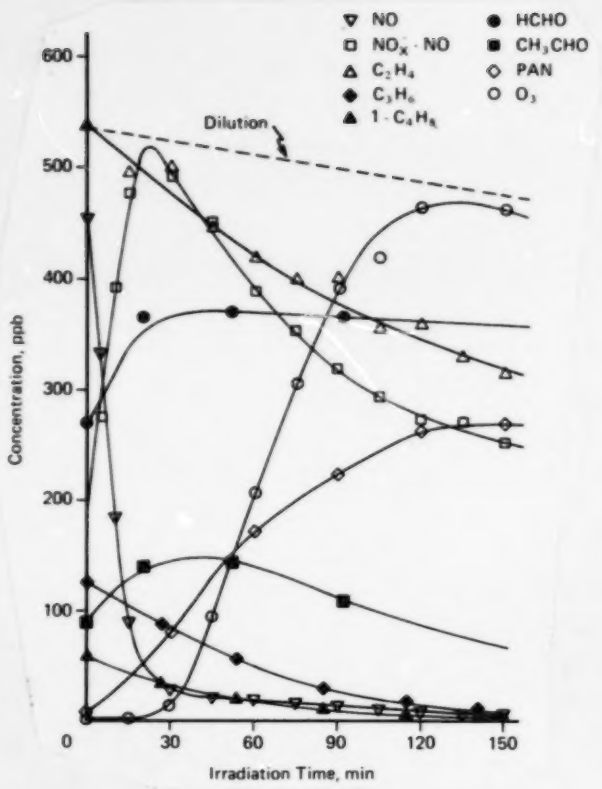

Figure 3. **Wood smoke static irradiation with 500 ppb additional NO_x.** Selected product and reactant profiles.

The particle size and volume distributions from the EAA exhibited the same characteristics as in the experiments without added NO_x. These data are shown in Figures A-2 and A-6 (Expt. B) and Figures A-3 and A-7 (Expt. C). For these experiments, the maximum in the volume distribution increased from 0.2 μm before irradiation to 0.4 μm following irradiation. The increase in the total volume of particulate matter as seen in Table 2 is two to three times greater than in Expt. A, a reflection of the larger extent of product formation with added NO_x.

Metals analysis was also performed on the particulate samples. The experiments with added NO_x represent a worst case for establishing whether photooxidation reactions may in some way affect the K/Fe ratio observed in wood smoke. Wood smoke K/Fe ratios ranging from 15 to 230 (mass basis) have been reported (14). For the determination reported here, a separate experiment having initial conditions similar to those for Experiments B and C was conducted. The filter sample taken before irradiation yielded K and Fe values of 17.1 mg/g and 0.76 mg/g, respectively. Following irradiation, K and Fe values of 4.24 mg/g and 0.28 mg/g, respectively, were measured. The smaller values following irradiation reflected an increase in the relative mass of the particulate due to adsorption from the gas phase, while the total mass of metal in the individual particles remained constant. Thus we obtain K/Fe ratios of 23 before irradiation and 15 following irradiation. The largest source of error in these determinations is from the Fe concentrations, which have an estimated accuracy of no better than ±50%. Thus, there appears to be little difference in the K/Fe ratio following irradiation, as anticipated.

The mutagenic activity exhibited in strains TA100 and TA98 from the exposure to the gas phase species in Experiments B and C are given in the lower half of Table 3. In all cases, the clean air and ambient air revertant levels were (within experimental error) identical to the spontaneous laboratory controls (TA100: 202 revertants/plate; TA98: 36 revertants/plate). The exposure time for each plot has been corrected as previously described. In Experiment B, data for an exposure time of 10 h (uncorrected) were also obtained. However, the irradiated mixture was toxic as indicated by depressed revertant levels in the survivor plates. For all other exposure times, no toxic effects were observed. The dose-response data (Figures B-5 and B-6) show systematically higher revertant levels for Experiment B, which can be attributed in part to the relatively greater extent of HC conversion for this experiment, as seen in Table 1.

The mutagenic activity exhibited with strains TA100 and TA98 for the particulate organic phase is also presented in Table 3. As in the case without added NO_X, the extracts from the products show no increased activity over that of the reactants in TA100 as seen in Figure B-7. Only in TA98 (Figure B-8) do the product extracts show significantly higher mutagenicity than do the reactants (approximately a factor of three). These particulate data are a compilation of filter extracts for all wood smoke/NO_X (added NO_X) irradiations performed during this study. The straight lines drawn through the data represent a least squares fit to all the data.

We also performed exposures of the gas phase reactants and products and plate incorporation tests for extracts using TA100 and TA98 with metabolic activation (S9). The gas phase revertant and product data are given under Experiment D in Table 1. The chemical analysis for the particulate data is given in Table 2. The number and volume distributions for the particulate matter are presented in Figures A-4 and A-8, respectively. The mutagenic activities of both phases are summarized in Table 4, and the individual dose-response curves are presented in Appendix C.

TABLE 4. MEASURED MUTAGENIC ACTIVITIES WITH S9 METABOLIC ACTIVATION FOR THE GAS AND PARTICULATE PHASES OF IRRADIATED WOOD SMOKE[a]

	Gas (revertants/h)		Particulate (revertants/μg)	
	TA100	TA98	TA100	TA98
Combustion NO_x				
Reactants	–	–	0.40 ± 0.19	0.35 ± 0.12
Products	–	–	0.19 ± 0.17	0.40 ± 0.10
Added NO_x				
Reactants	3.1 ± 1.7[b]	−2.8 ± 0.9[b]	0.23 ± 0.04	0.28 ± 0.06
Products	122 ± 8	20 ± 7	0.25 ± 0.11	0.82 ± 0.26

a Values represent slopes from the dose-response curves. The uncertainty given is the 95% confidence interval.
b Survivor data indicate no toxicity. Slight positive or negative response probably a reflection of the large relative uncertainty with a small response.

SECTION 5

DISCUSSION

Under the conditions of these experiments, the irradiation of wood smoke/NO_X mixtures dramatically increases the mutagenic activity, particularly for the gas phase component. As noted earlier, the integrated light intensity and the hydrocarbon/NO_X ratio are not unreasonable for actual wintertime conditions. The wood smoke gas phase mixture consists of a wide variety of reactive species including alkenes, aromatics, aldehydes, and oxygen heterocycles. From examination of Figures 2 and 3 and Table 1, this clearly is a highly reactive system. For Experiment B, with added NO_X, at least 2.4 ppmC of reactants were converted to products. In addition, the observed decrease in the particulate phase species concentrations (Table 2) indicates that considerable particulate phase reaction is occurring. From the data in Table 1, it can be seen that although we have identified a large number of gas phase reactants, we have not accounted for ~50% of the measured total HC level. A detailed analysis of the total observed mutagenic activity would require an extensive analysis of minor products and measurement of their individual mutagenic activities. We have found, for example, that the mutagenic activity of irradiated toluene/NO_X and propylene/NO_X mixtures is apparently due largely to the presence of minor products (10, 11). In addition, although detailed reaction mechanisms have been formulated (and summarized by Atkinson and Lloyd [18]) for the photooxidations of a wide variety of HCs, large uncertainties exist, particularly for aromatic species such as toluene. Considering that the well-known aromatic ring fragmentation products glyoxal and methyl glyoxal (which are very reactive to OH) either increase or do not significantly decrease during the irradiation, it would appear that a significant degree of aromatic photooxidation is occurring. It therefore is apparent that an analysis of these results, based on mutagenic contributions from individual products, is not feasible for this complex mixture.

In this discussion, we focus on comparing the relative mutagenic activities of the sum of all gas phase reactants and products to the particulate phase reactants and products. To achieve this comparison, both the mutagenic activity and corresponding dose for each phase must be determined. The particulate phase dose using the plate incorporation test is completely straightforward (i.e., a known weight of the extract is added to the plates). To determine the gas phase dose, the total quantity of gas phase species that deposits into the test medium during the exposure must be measured. In previous studies involving propylene and toluene, we have found that the deposition for pure HCs (containing only carbon and hydrogen) is extremely low (<1% of the total gas phase mass). On the other hand, polar compounds such as formaldehyde are generally

very soluble. Thus, the more polar products tend to dissolve into the medium, whereas reactant HCs do not. We measured the total HC concentration in the effluent (product) biochamber both before and after the plates were opened using the total HC analyzer. (Wall loss of gas phase species in the exposure chambers is negligible as determined from the total HC.) The decrease in the total gas phase HC concentration can be related to the total mass of material deposited into the plates. We made measurements of the HC decrease for the two experiments with added NO_X and have obtained an average value of 1.36 ppmC. For the experiment with combustion NO_X only, the amount of conversion of reactants to gas phase products was considerably less, and an accurate measurement of the decrease in HC when the plates were opened was not attainable. In addition, since the revertant responses were much greater in the experiments with added NO_X, their measurement could be made with greater accuracy. Also, for exposures up to 10 h and extract doses up to 850 µg/plate, no significant difference in mutagenic activities was observed for the addition of S9 for either reactants or products in either strain. Therefore, the balance of this discussion focuses on the results from the experiments with added NO_X and without metabolic activation.

With this datum (i.e., ΔHC = 1.36 ppmC) and the use of two assumptions, we can estimate the mass of gas phase material deposited into each plate. The method (and equations) is analogous to those previously described (11), except that this calculation is performed for the sum total of products. Given 1.36 ppmC measured deposition into the plates and assuming the average total HC analyzer sensitivity to the deposited products is the same as for acetone (71% that of C_3H_8), we calculate that 1.11 µmoles carbon has deposited into each plate per hour of exposure. From the products columns for Experiments B and C of Table 1, an average mass of 18.5 g associated with each mole of carbon is calculated. Using this value, a deposition rate of 20.5 µg plate^{-1} h^{-1} is obtained for gas phase products. (The sensitivity of the THC analyzer to acetone was measured in a separate experiment using a known gas phase concentration of acetone. The response was 71% of that obtained when measuring the same concentration of propane. Acetone was selected because its average molecular weight per carbon atom, 19.3 g/mole, is nearly the same as the average molecular weight per carbon of the measured gas phase product constitutents in Table 2, 18.5 g/mole.) We are now able to estimate the mutagenic activity of the gas phase species on a per mass basis. Table 3 shows mutagenic activities of 174 revertants plate^{-1} h^{-1} for TA100 and 30.3 revertants plate^{-1} h^{-1} for TA98 (as averages for the two experiments). By dividing these values by the calculated deposition for the gas phase products, activities of 8.5 and 1.5 revertants/µg are obtained for TA100 and TA98, respectively. These values represent a reasonable estimate of the mutagenic activity of the species that are actually transported into the test medium. If it were assumed that the undissolved materials were also equally mutagenic, then these values would represent a reasonable estimate of the mutagenic activity per microgram of the total gas phase product mixture. The last assumption is

probably not valid, because the highly polar products are more likely to dissolve into the medium, and these components also tend to be more mutagenic (9-11). It is more reasonable, therefore, to consider the activities of 8.5 and 1.5 revertants/μg as estimates of the upper limit of the mutagenic activity of the total gas phase effluent mixture.

A lower limit for the mutagenic activity per microgram can be calculated by assuming that all the undissolved gaseous materials are completely nonmutagenic. The dissolved and undissolved components taken together represent the total gas phase HCs shown in Table 2. (Since the majority of the total effluent HC is probably unreacted, saturated HCs, an instrumental sensitivity equivalent to that for propane has been used in this calculation.) Using the average value of 14.1 ppmC from Experiments B and C, lower limit values of 1.2 and 0.20 revertants/μg are calculated for TA100 and TA98, respectively.

The true mutagenic activity is probably intermediate between the values cited above. Nonpolar compounds are probably not completely nonmutagenic, nor is it reasonable to assume that all of the polar, gas phase mutagenic materials are removed under these experimental conditions. For example, under these experimental conditions, 15% of the PAN (which is polar and mutagenic) is deposited into the biological assay (10). The undissolved products are, therefore, likely to have some mutagenic potential, although presently there is no best estimate for their contribution to the mutagenic activity without invoking severe assumptions. The calculated range of values is given in Table 5.

TABLE 5. COMPARISON OF THE GAS AND PARTICULATE PHASE MUTAGENIC ACTIVITY (REVERTANTS/μg) FOR WOOD SMOKE AND IRRADIATED WOOD SMOKE, STRAINS TA100 AND TA98 WITHOUT METABOLIC ACTIVATION

	TA100		TA98	
	Gas	Particulate	Gas	Particulate
Reactants	0	0.3	0	0.2
Products	1.2 - 8.5	0.3	0.2 - 1.5	0.9

The values for the gas phase reactants are more uncertain because the deposition into the plates was not measured. For both strains, the mutagenic activity (revertants h^{-1}) is zero within the experimental error. Since the reactants do contain appreciable quantities of polar species, such as HCHO, the deposition, though lower than 1.36 ppmC, is probably significant. Therefore, it is reasonable to assume the gas phase reactant mutagenic activities to be 0 revertants/μg for both strains.

The data for the mutagenic activity on a per mass basis for both phases have been summarized in Table 5. The particulate phase activity is obtained directly from the data in Table 3 for both

reactants and products. For TA98, the particulate phase and gas phase reactants show low activity, whereas the products in both phases show significant mutagenic activity. As we have stated, the volume (and thus mass) of the particulate matter increases considerably during irradiation, probably because of absorption of gas phase reaction products. Since the particulate phase mutagenicity increases for TA98, these gas phase condensible compounds may be significantly mutagenic, at least for this strain. For TA100, only the gas phase products show substantial mutagenic activity.

It should be noted that the slopes of the dose-response curves (Table 3) for Experiment A (combustion NO_X only) are several times smaller (factors of 5 and 9 for TA100 and TA98, respectively) than those for the experiments with added NO_X. In Experiment A, the conversion of gas phase HCs is approximately a factor of two less, and therefore the total deposition and observed mutagenic activity might be expected to be lower by roughly this amount. If, however, some of the mutagens present are nitrated species, then the relative mutagenic activity of the gas phase products may be lower in Experiment A since less NO_X is available for the production of nitrates.

The mutagenic activity data of the particulate phase with TA98 generally agree with the data of Kamens *et al.* (7). In their work, the reactant particulate matter consistently yielded mutagenic activities of 0.1 to 0.3 revertants/µg (-S9). For the dark oxidation of wood smoke particulate matter by O_3 and NO_2, mutagenic activities ranging from 0.4 to 2.3 revertants/µg were observed for a wide variety of reaction conditions. This activity is on the same order (within a factor of two) of the response observed in this study when wood smoke was irradiated in the presence of added NO_X. In addition, they did present one set of experiments in which an irradiation was performed using combustion NO_X (50 ppb) only. The mutagenic activity of the particulate phase products rose only slightly, from 0.18 to 0.23 revertants/µg. These results are very similar to our results for the experiment with combustion NO_X only, in which the particulate phase mutagenic activity (TA98) rose from 0.20 to 0.26 revertants/µg following irradiation, although that increase is within the stated uncertainties.

The analysis up to this point gives only the mutagenic activities (revertants/µg) and does not take into account the relative amounts of material present in the two phases. However, it is clear that for TA100 the only significant source of mutagenic activity arises from gas phase products. It should be noted that in general, TA100 is believed to be relatively more sensitive to low molecular weight polar mutagens, and TA98 is more sensitive to high molecular weight species, such as PAHs.

An alternative means for summarizing these results is to calculate the total mutagenic activity for the two phases in terms of revertants/m^3. In this way, the values obtained will also reflect the relative amounts of material, as well as their inherent mutagenic activity, present in each phase. It should be noted that the relative quantities of particulate and gas phase emissions are a complex

function of burn conditions and wood type. Ramdahl *et al.* (4) indicated that burn conditions appear to be the major factor determining emissions, far outweighing the dependence on wood type. Dasch (3) has found that under fast burn conditions there is relatively less particulate mass than under slow burn conditions. In preparing the reactant system during this study, we introduced wood smoke into the reaction chamber over an extended period (12 h) to integrate transient effects of burn conditions. The total mutagenic activity (revertants/m^3) for the gas and particulate phases is calculated by multiplying the mutagenic activity (revertants/µg) by the gas and particulate phase concentrations (µg/m^3). The results of these calculations are shown in Table 6. The ranges for the gas phase products correspond to the upper and lower limits given in Table 5.

TABLE 6. COMPARISON OF THE GAS AND PARTICULATE PHASE TOTAL MUTAGENIC (REVERTANTS/m^3) FOR WOOD SMOKE AND IRRADIATED WOOD SMOKE, STRAINS TA100 AND TA98 WITHOUT METABOLIC ACTIVATION

	TA100		TA98	
	Gas	Particulate	Gas	Particulate
Reactants	0	100	0	80
Products	12,300 - 90,600	180	2,130 - 16,000	730

The particulate (and gas) phase activity for the products could conceivably be low (in terms of revertants/m^3) because of wall loss during the irradiation. The wall loss rate for the particulate phase has been measured in a separate experiment in which the wall loss rate was determined using the EAA. Determining the wall loss rate using the EAA is valid if we assume that the particles are in the range of 0.01-1 µm and that the density of the particles remains constant with time. In the absence of any photochemical reaction, this second assumption is expected to hold. Gravimetric analysis was not used because its precision is not as good as that of the EAA unless very large samples are acquired, which would tend to limit severely the number of available samples. The wall loss experiment indicated an overall rate of disappearance for particles (0.01-1 µm) of 4-5% h^{-1}, which translates to a 10% loss of particulate matter to the walls during a 2.5-h irradiation. The particulate phase wall loss after the irradiation (during sample collection) would have no effect on the calculation of mutagenic activity in terms of revertants/m^3 because the result is obtained by multiplying the particulate mass concentration (µg/m^3) at the end of the irradiation by the mutagenic activity in revertants/µg. The only conceivable impact would be if particles of a particular diameter and mutagenic activity significantly different from the average were preferentially lost to the walls. It seems reasonable to assume for these calculations that this is not the case. Thus, a correction to account for the wall loss is not expected to increase significantly the total mutagenic activity of the particulate phase.

Wall losses for gas phase products could also be important, especially for polar compounds, e.g., organic hydroperoxides or nitrates. Such a wall loss would decrease the mutagenic activity with time. However, if gas phase losses were important, negative curvature would be seen in Figure B-1, for example. A wall loss experiment using the total HC analyzer indicated that wall losses over the time scale of these exposures would not be significant. In addition, although dark gas phase reactions could produce or remove mutagenic compounds during the course of the exposure, the linear dose response curves argue against this occurrence.

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APPENDIX A

EAA NUMBER AND VOLUME DISTRIBUTIONS

The number distribution for Experiments A, B, C, and D are shown graphically as bar graphs in Figures, A-1, A-2, A-3, and A-4, respectively. The corresponding volume distributions are shown in Figures A-5 to A-8.

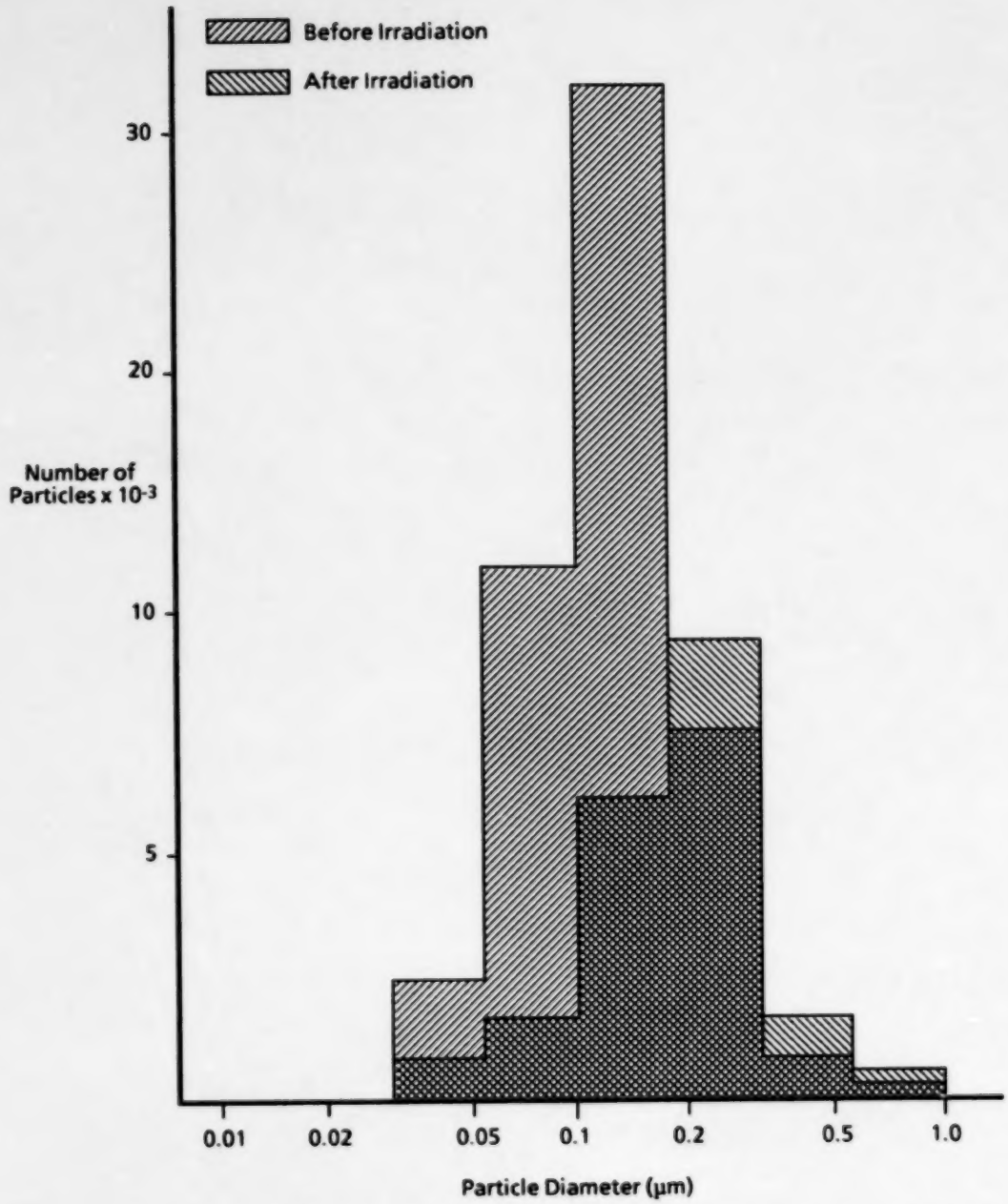

Figure A-1. EAA number distribution for diluted wood smoke before and following irradiation in Experiment A.

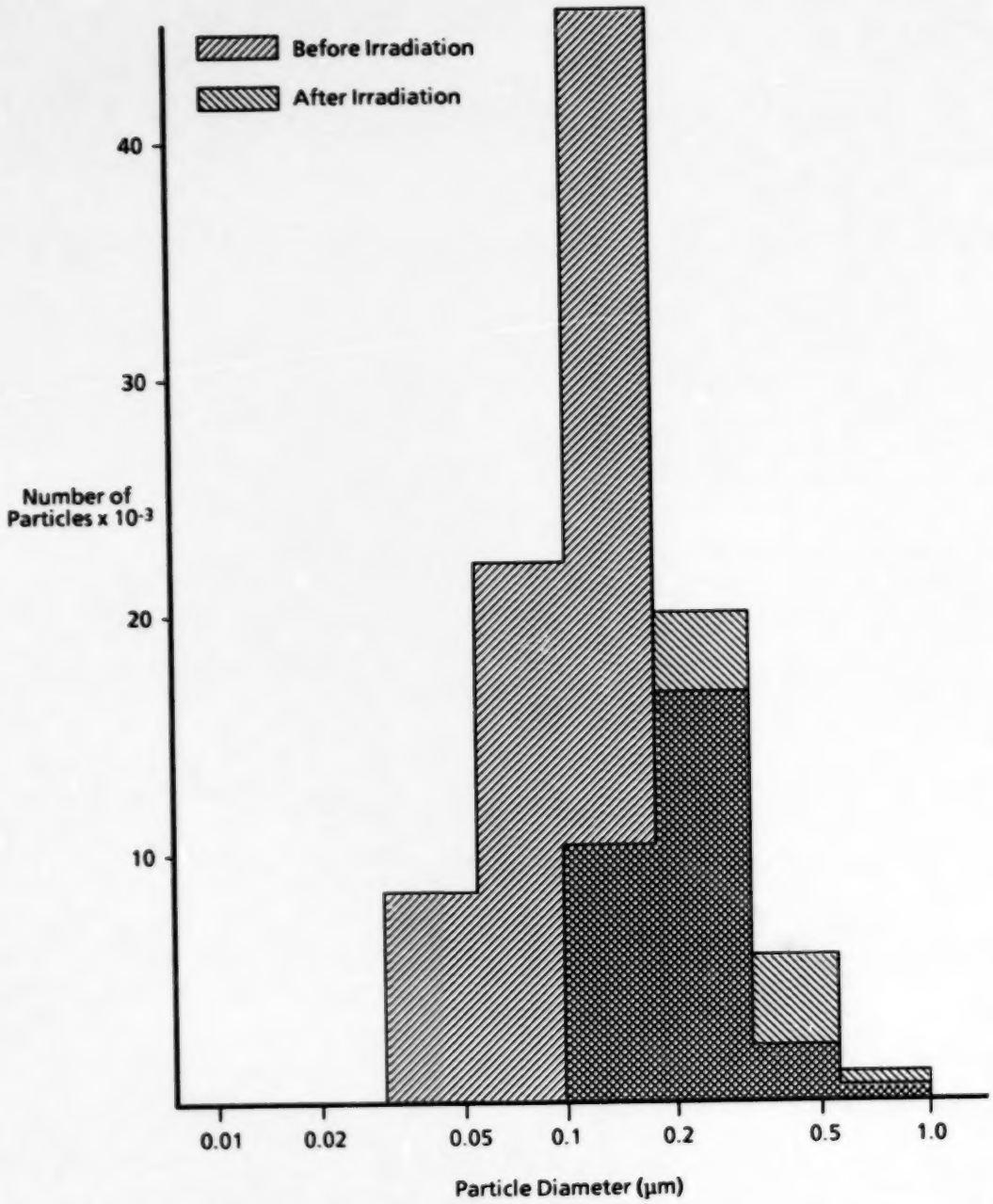

Figure A-2. EAA number distribution for diluted wood smoke before and following irradiation in Experiment B.

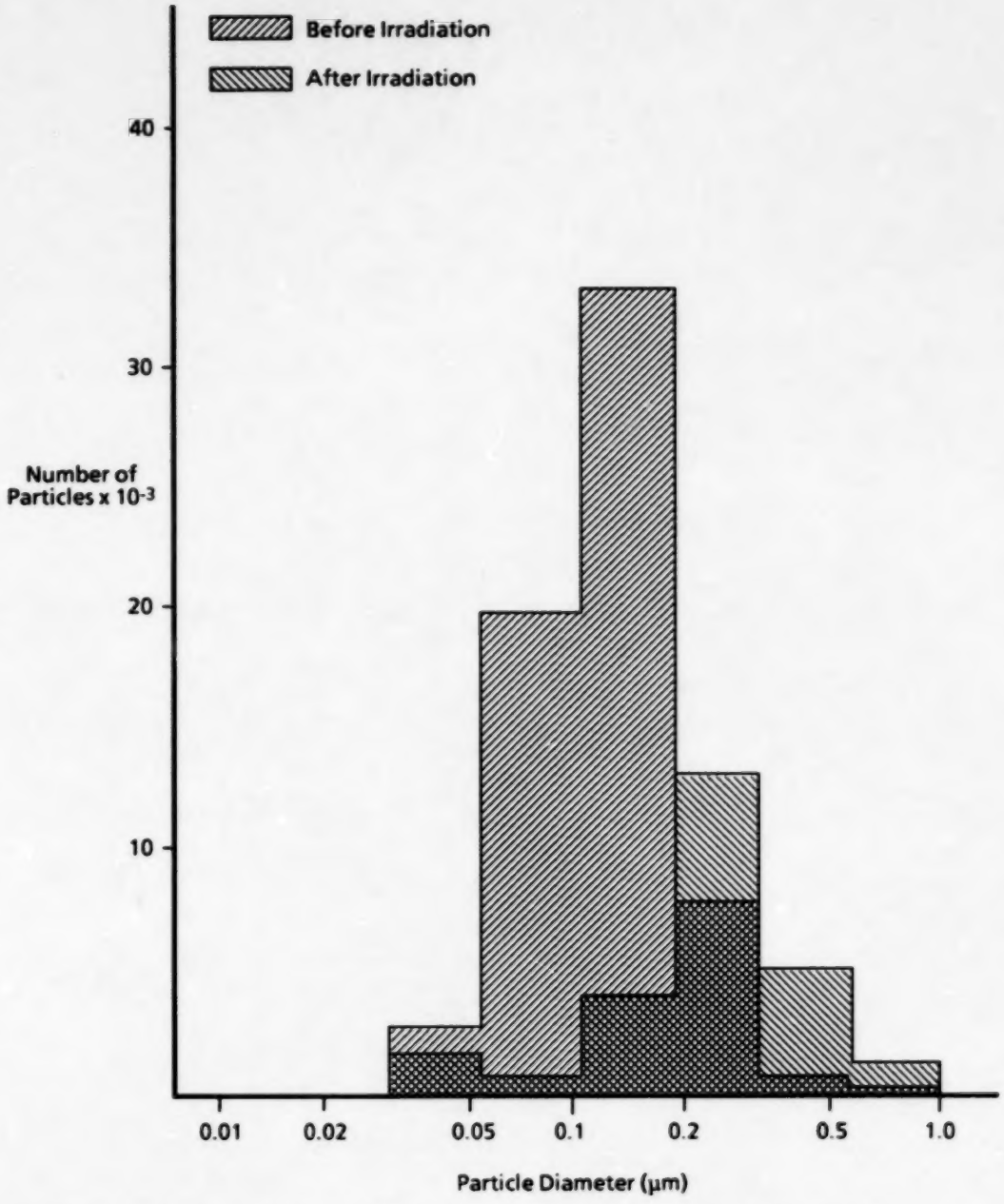

Figure A-3. EAA number distribution for diluted wood smoke before and following irradiation in Experiment C.

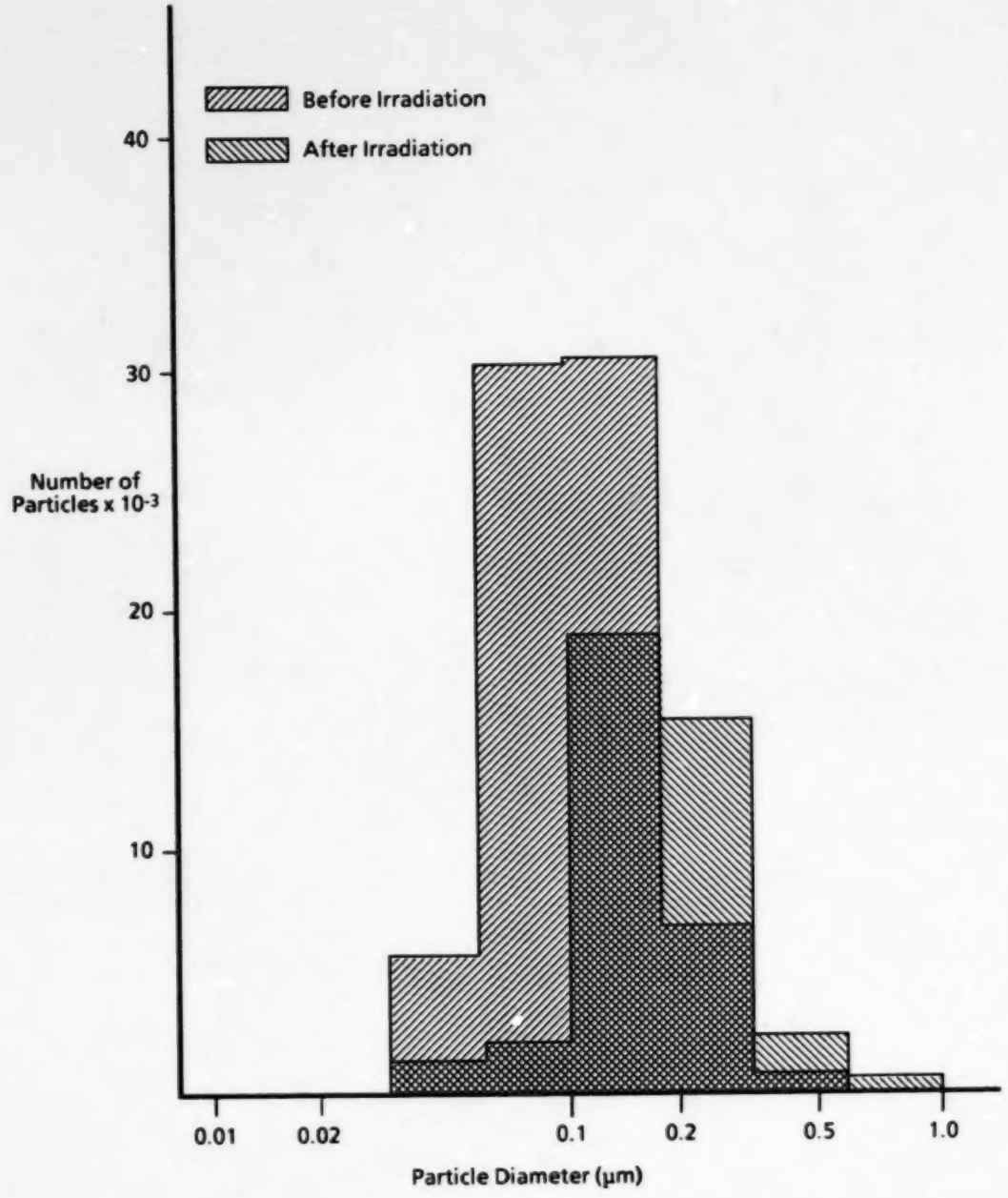

Figure A-4. EAA number distribution for diluted wood smoke before and following irradiation in Experiment D.

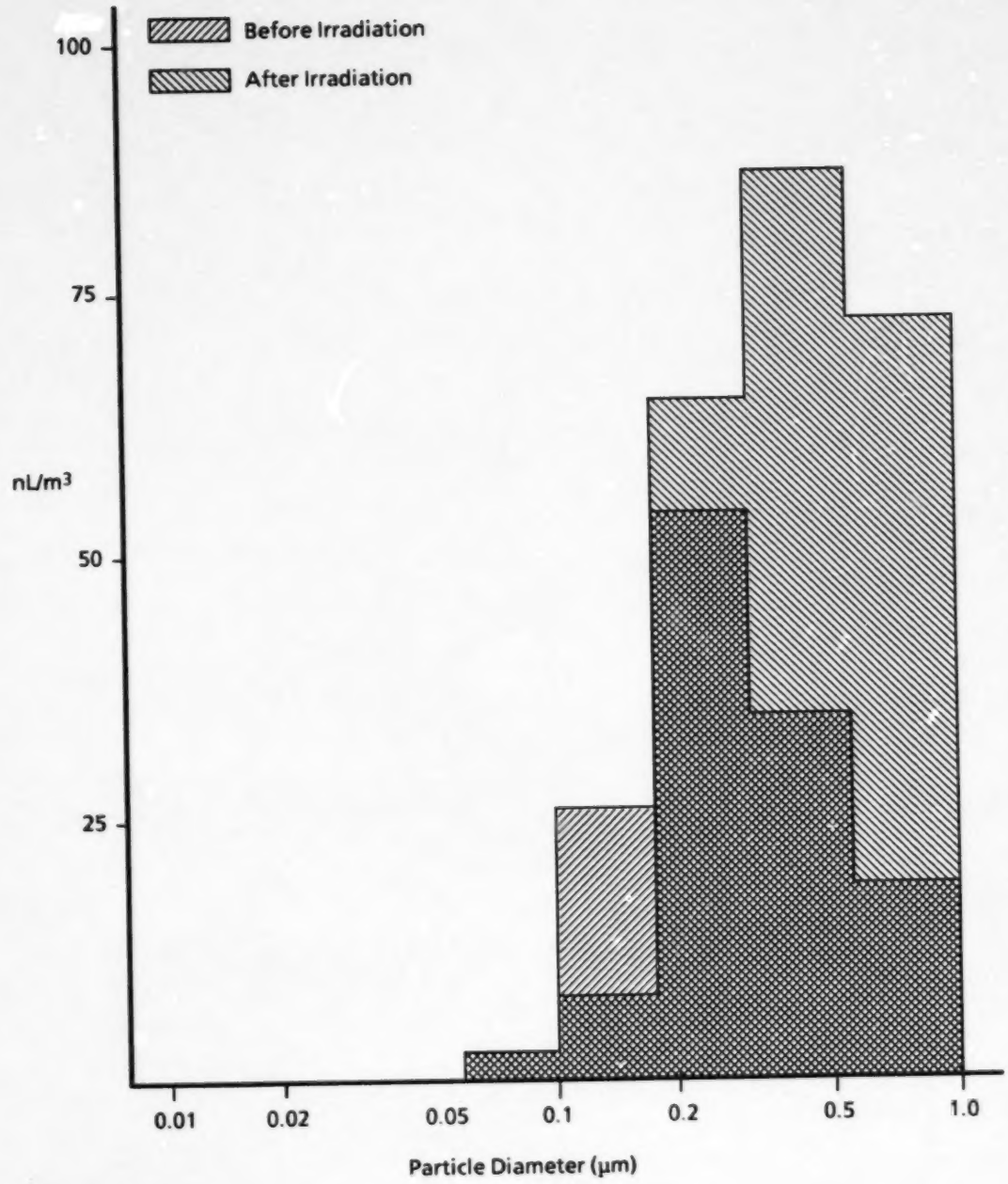

Figure A-5. EAA volume distribution for diluted wood smoke before and following irradiation in Experiment A.

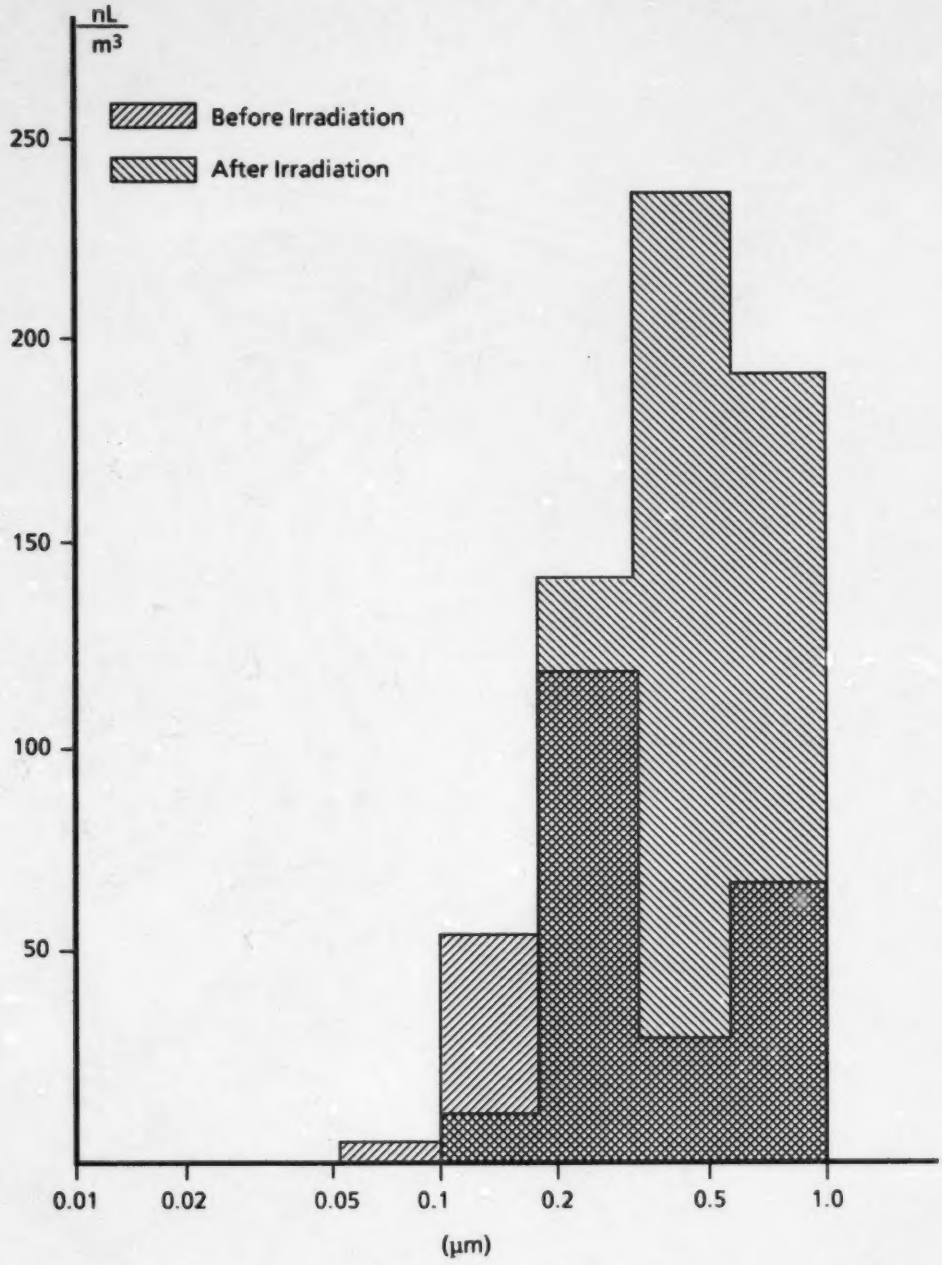

Figure A-6. EAA volume distribution for diluted wood smoke before and following irradiation in Experiment B.

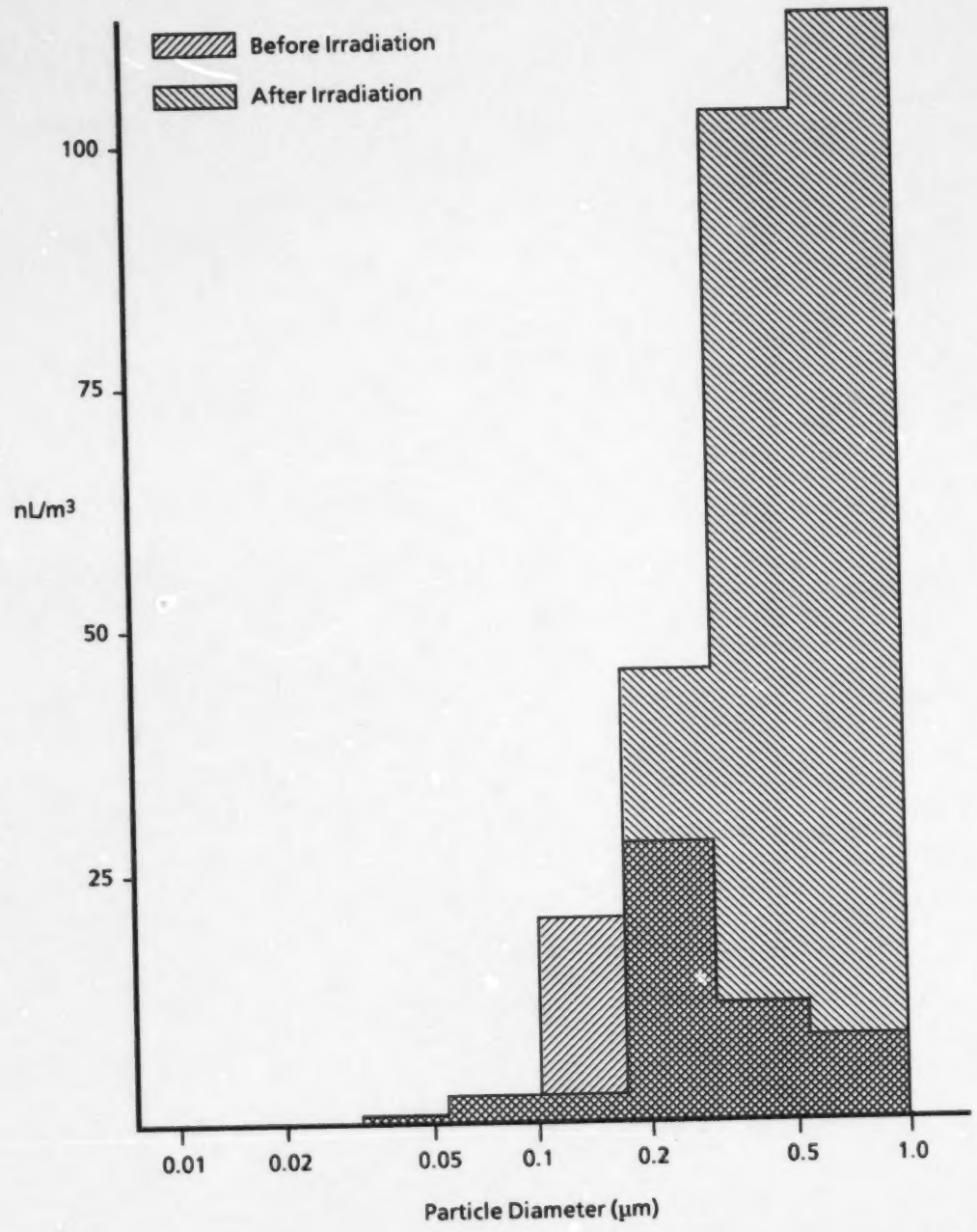

Figure A-7. EAA volume distribution for diluted wood smoke before and following irradiation in Experiment C.

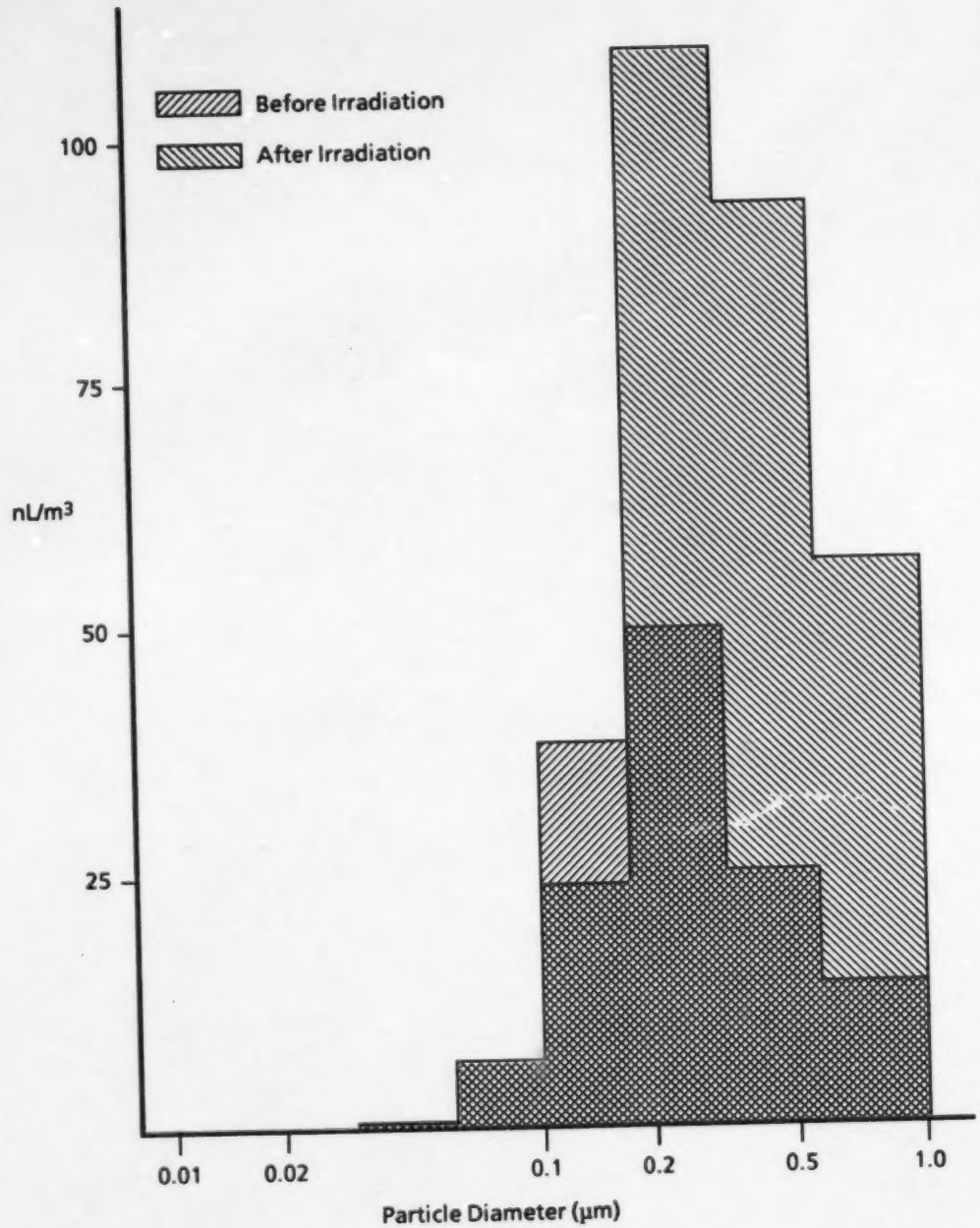

Figure A-8. EAA volume distribution for diluted wood smoke before and following irradiation in Experiment D.

APPENDIX B

DOSE RESPONSE CURVES FOR THE MUTAGENIC ACTIVITY OF WOOD SMOKE WITHOUT METABOLIC ACTIVATION

The individual dose response curves for the entries in Table 3 are given in this appendix. The arrangement of figures according to the figure number and table entry is given as follows.

	Gas (revertants/h)		Particulate (revertants/µg)	
	TA100	TA98	TA100	TA98
Combustion NOx				
Reactants	B-1*	B-2	B-3	B-4
Products	B-1	B-2	B-3	B-4
Added NOx				
Reactants	B-5	B-6	B-7	B-8
Products	B-5	B-6	B-7	B-8

*Figure B-1

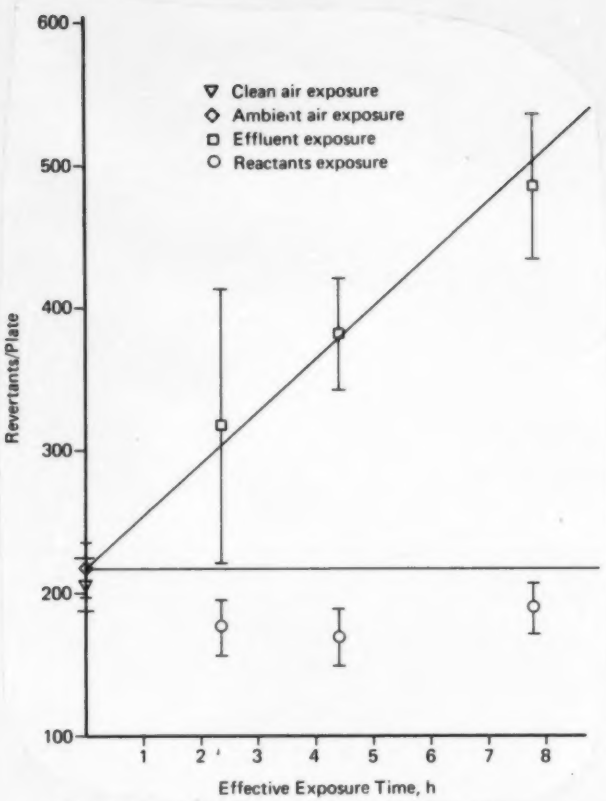

Figure B-1. Dose-response curve for the mutagenic activity (TA100) of the gas phase components of wood smoke before and after irradiation.

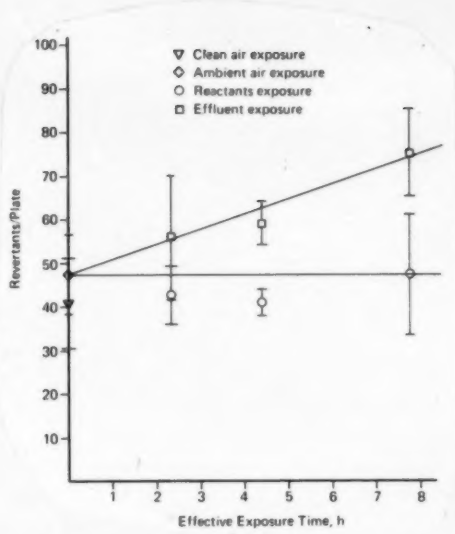

Figure B-2. Dose-response curve for the mutagenic activity (TA98) of the gas phase components of wood smoke before and after irradiation.

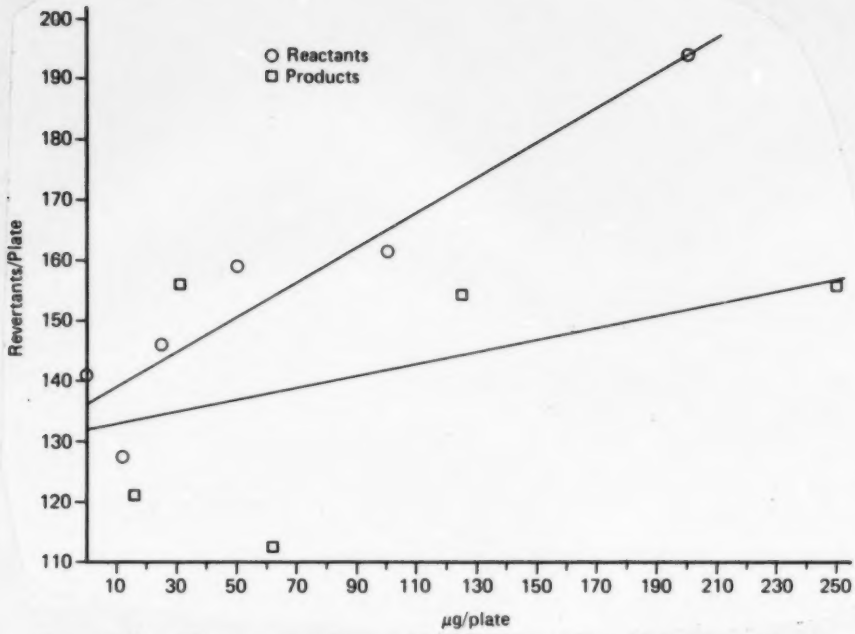

Figure B-3. Dose-response curve for the mutagenic activity (TA100) of the particulate extracts of wood smoke before and following irradiation.

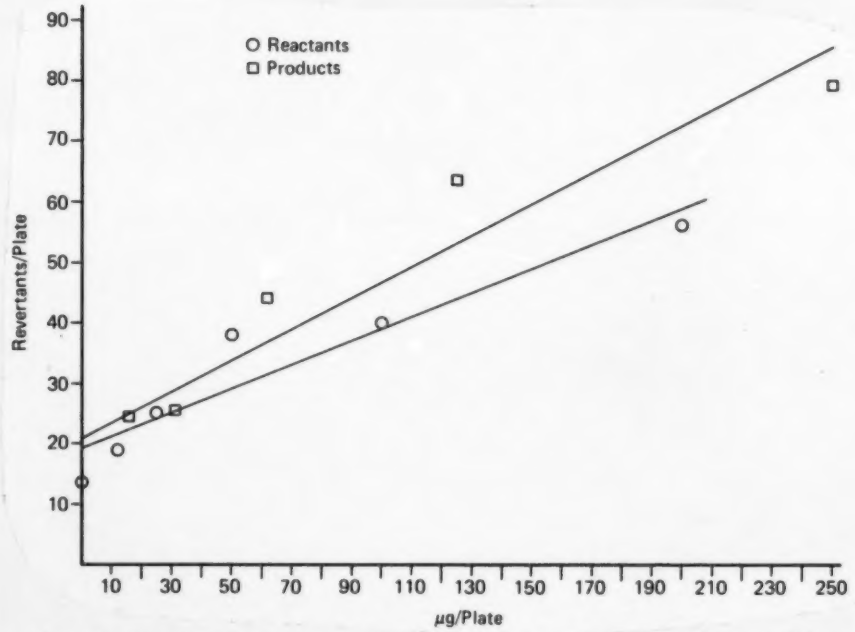

Figure B-4. Dose-response curve for the mutagenic activity (TA98) of the particulate extracts of wood smoke before and following irradiation.

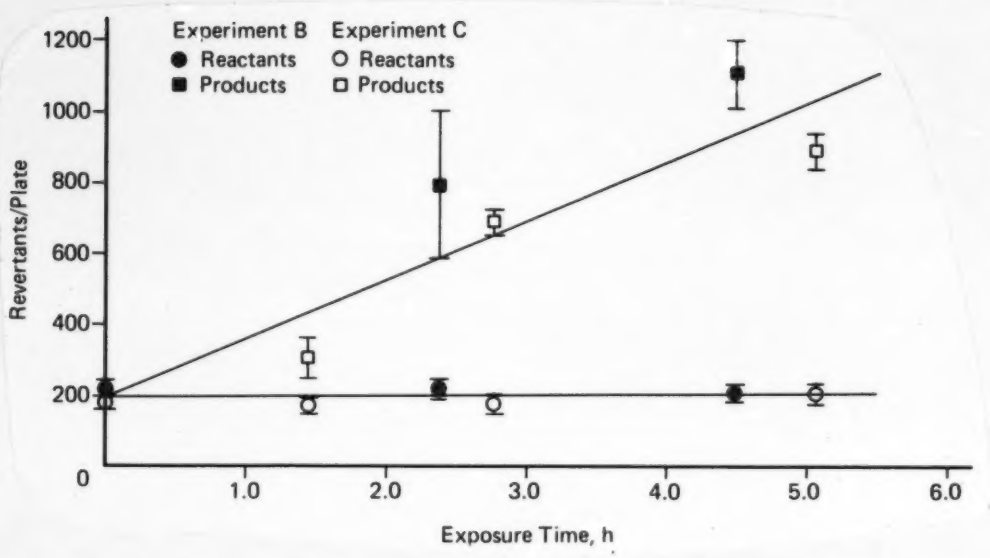

Figure B-5. Dose-response curve for the mutagenic activity (TA100) of the gas-phase components of wood smoke (+ 500 ppb NO_X) before and following irradiation.

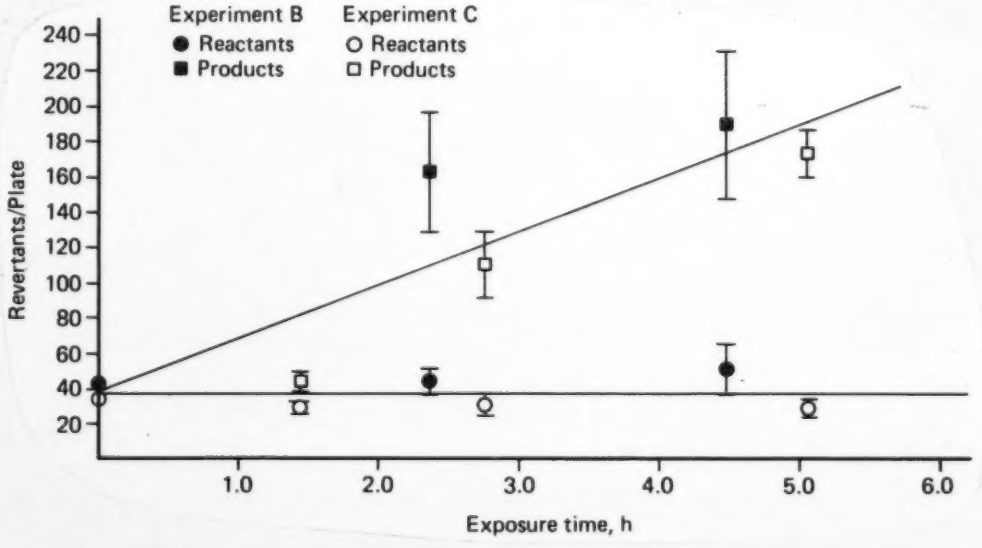

Figure B-6. Dose-response curve for the mutagenic activity (TA98) of the gas-phase components of wood smoke (+ 500 ppb NO_X) before and following irradiation.

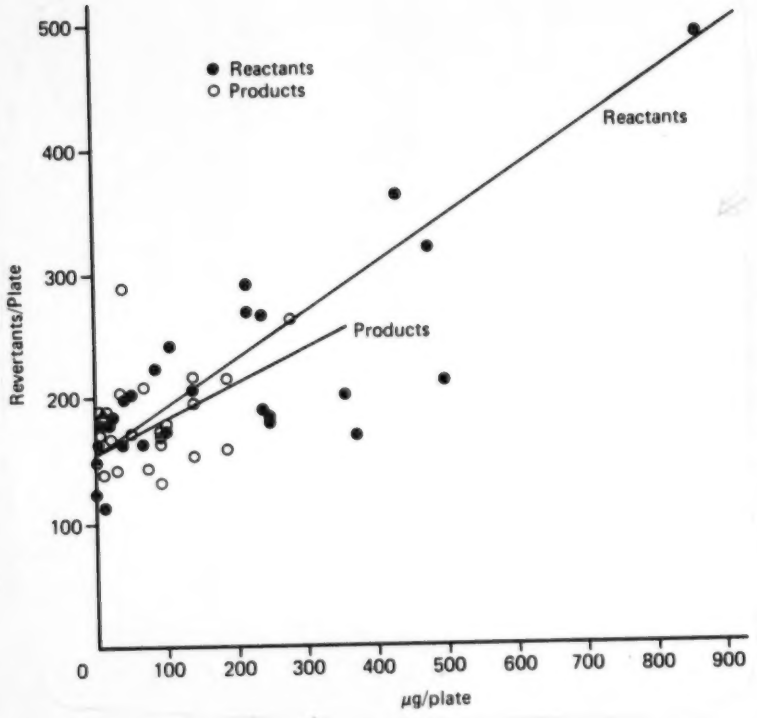

Figure B-7. Dose-response curve for the mutagenic activity (TA100) of the particulate extracts of wood smoke (+ 500 ppb NO_X) before and following irradiation.

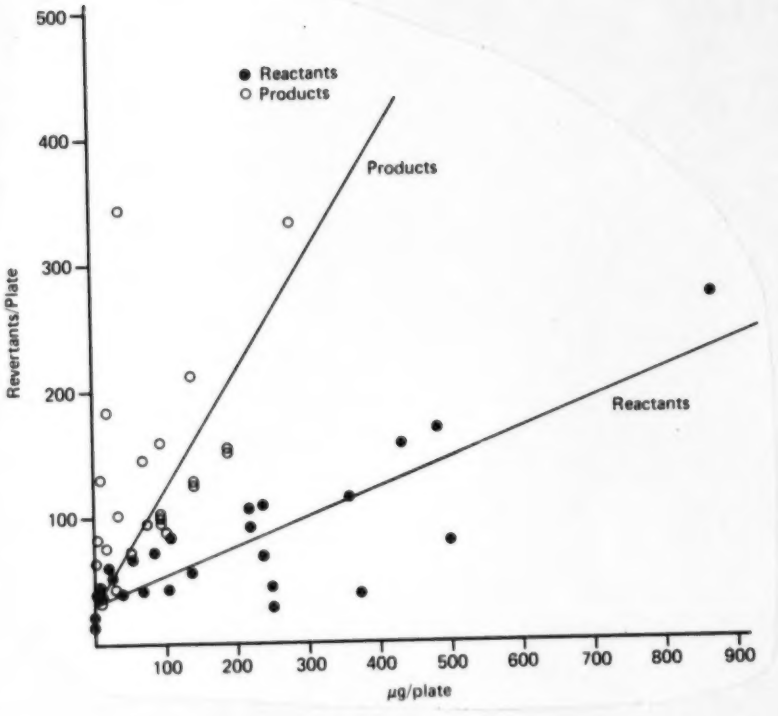

Figure B-8. Dose-response curve for the mutagenic activity (TA98) of the particulate extracts of wood smoke (+ 500 ppb NO_X) before and following irradiation.

APPENDIX C

DOSE RESPONSE CURVES FOR THE MUTAGENIC ACTIVITY OF WOOD SMOKE WITH METABOLIC ACTIVATION

The individual dose response curves for the entries in Table 4 are given in this appendix. The order of figures and corresponding table entry is given as follows.

	Gas (revertants/h)		Particulate (revertants/µg)	
	TA100	TA98	TA100	TA98
Combustion NOx				
Reactants			C-1	C-2
Products	–	–	C-1	C-2
Added NOx				
Reactants	C-3	C-4	C-5	C-6
Products	C-3	C-4	C-5	C-6

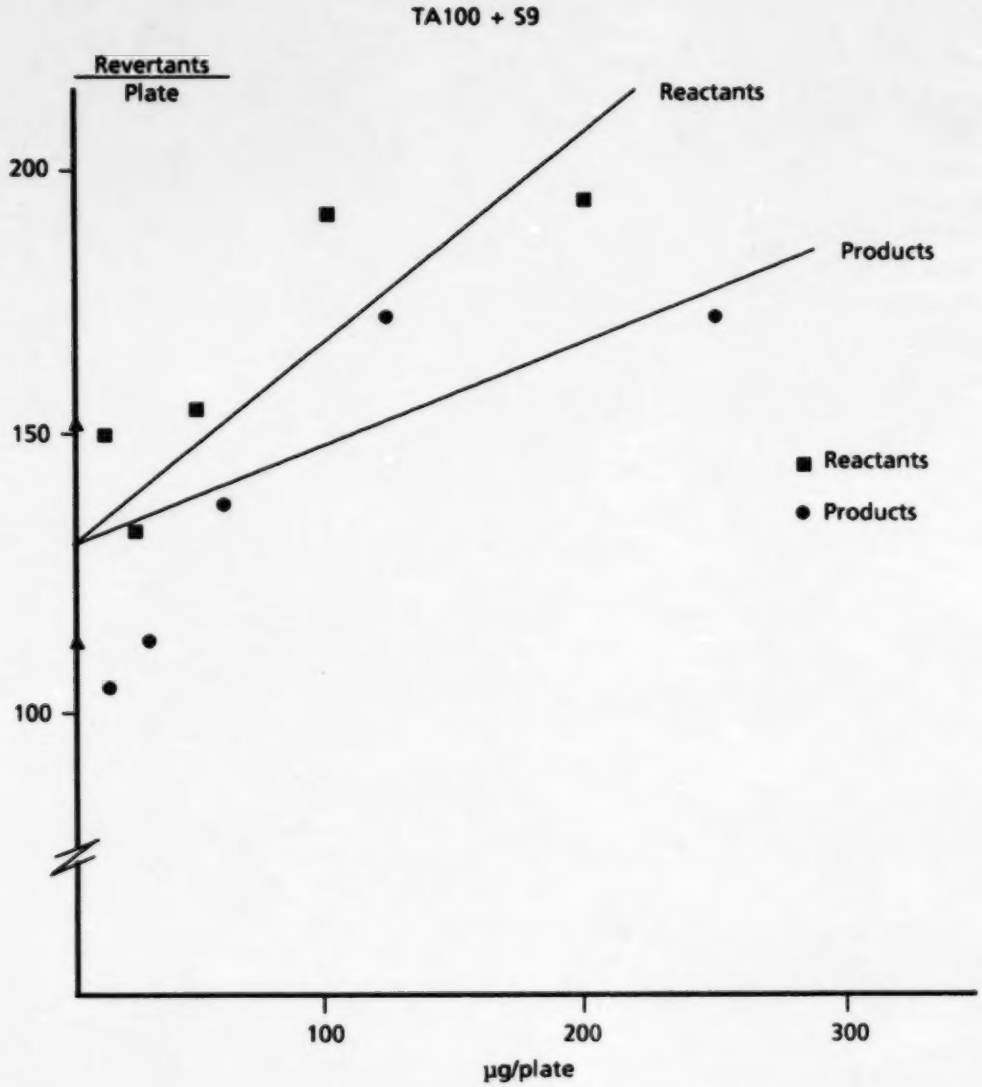

Figure C-1. Dose-response curve for the mutagenic activity (TA100 + S9) of the particulate extracts of wood smoke before and following irradiation.

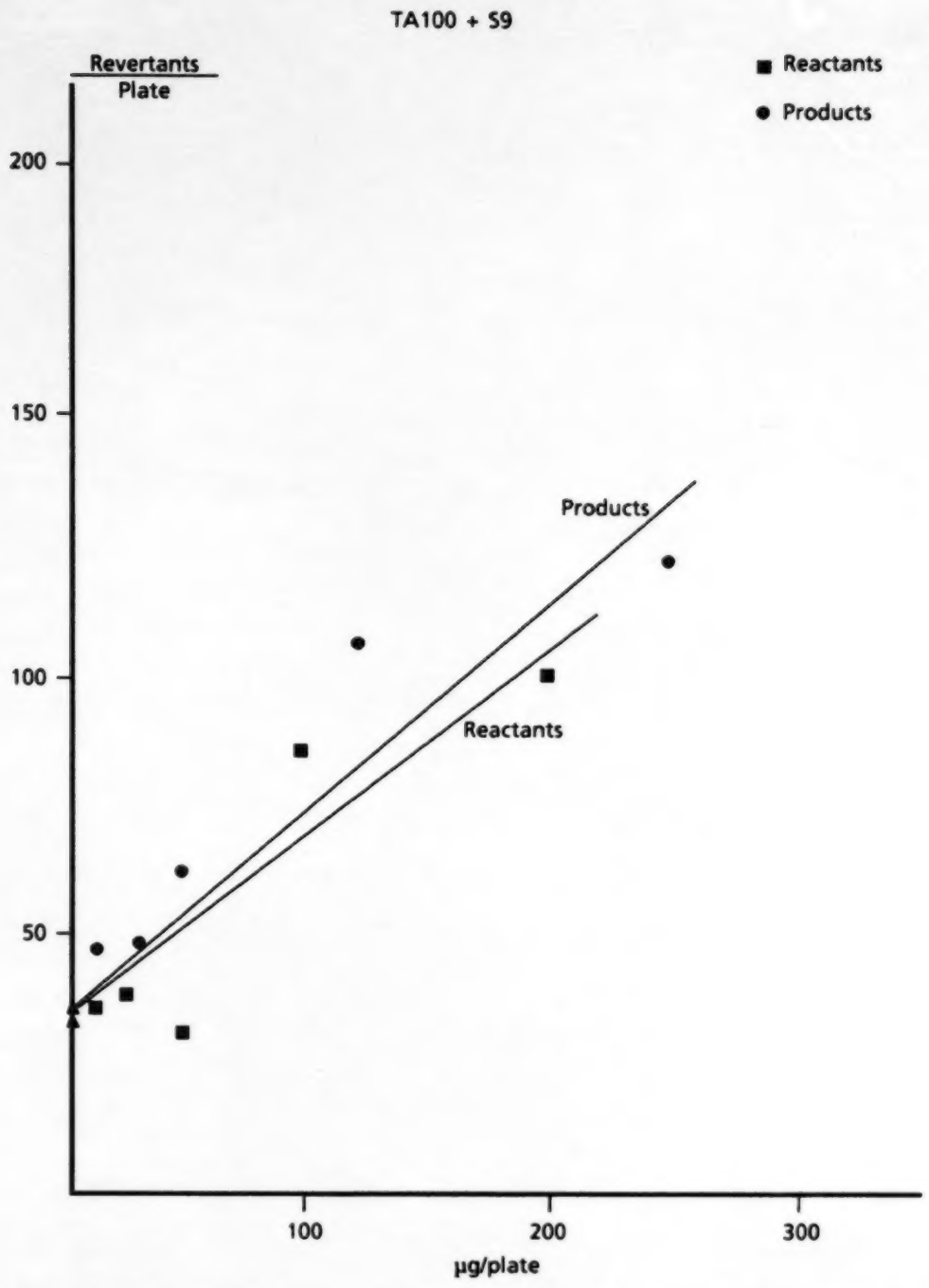

Figure C-2. Dose-response curve for the mutagenic activity (TA98 + S9) of the particulate extracts of wood smoke before and following irradiation.

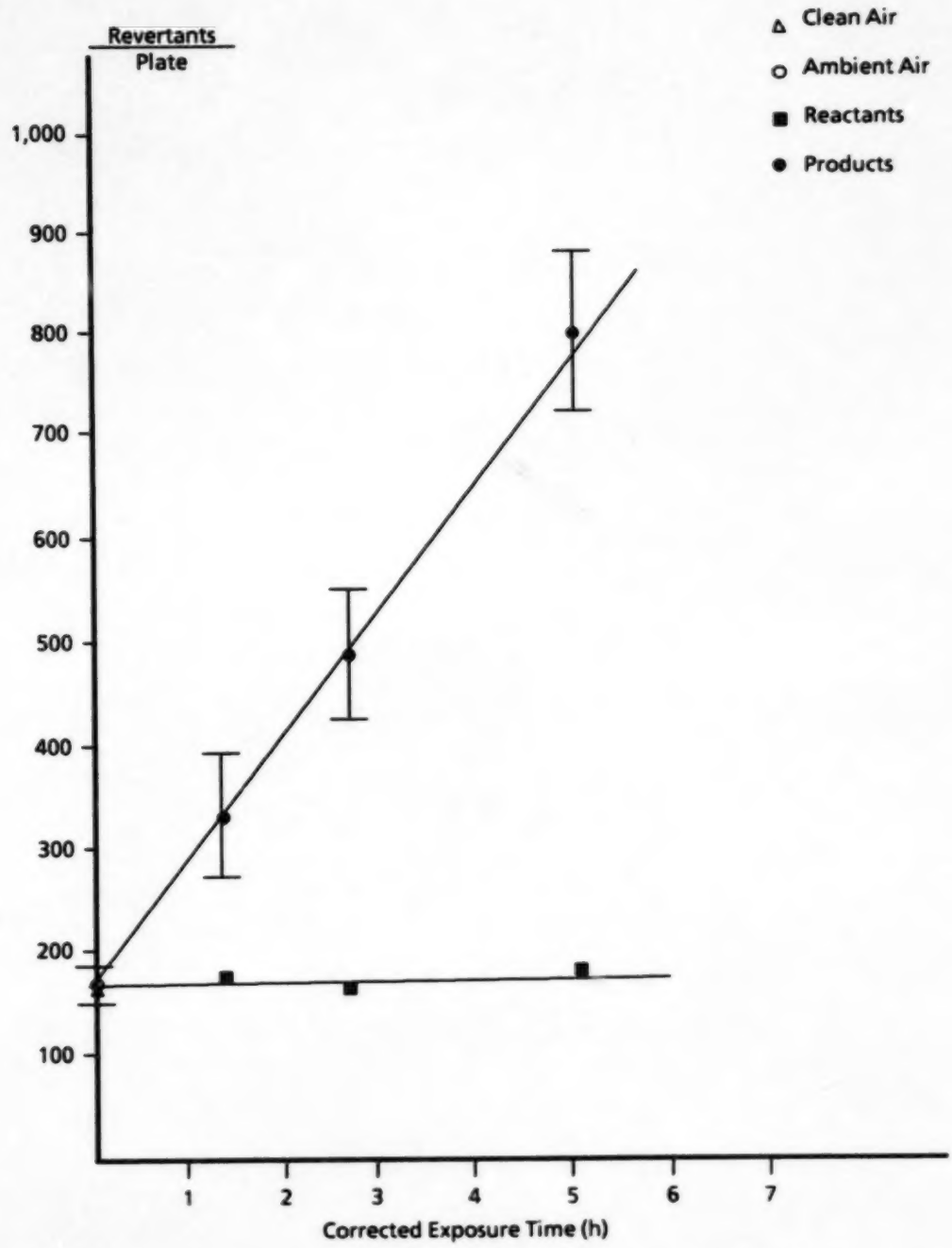

Figure C-3. Dose-response curve for the mutagenic activity (TA100 + S9) of the gas-phase components of wood smoke (+ 500 ppb NO_X) before and following irradiation.

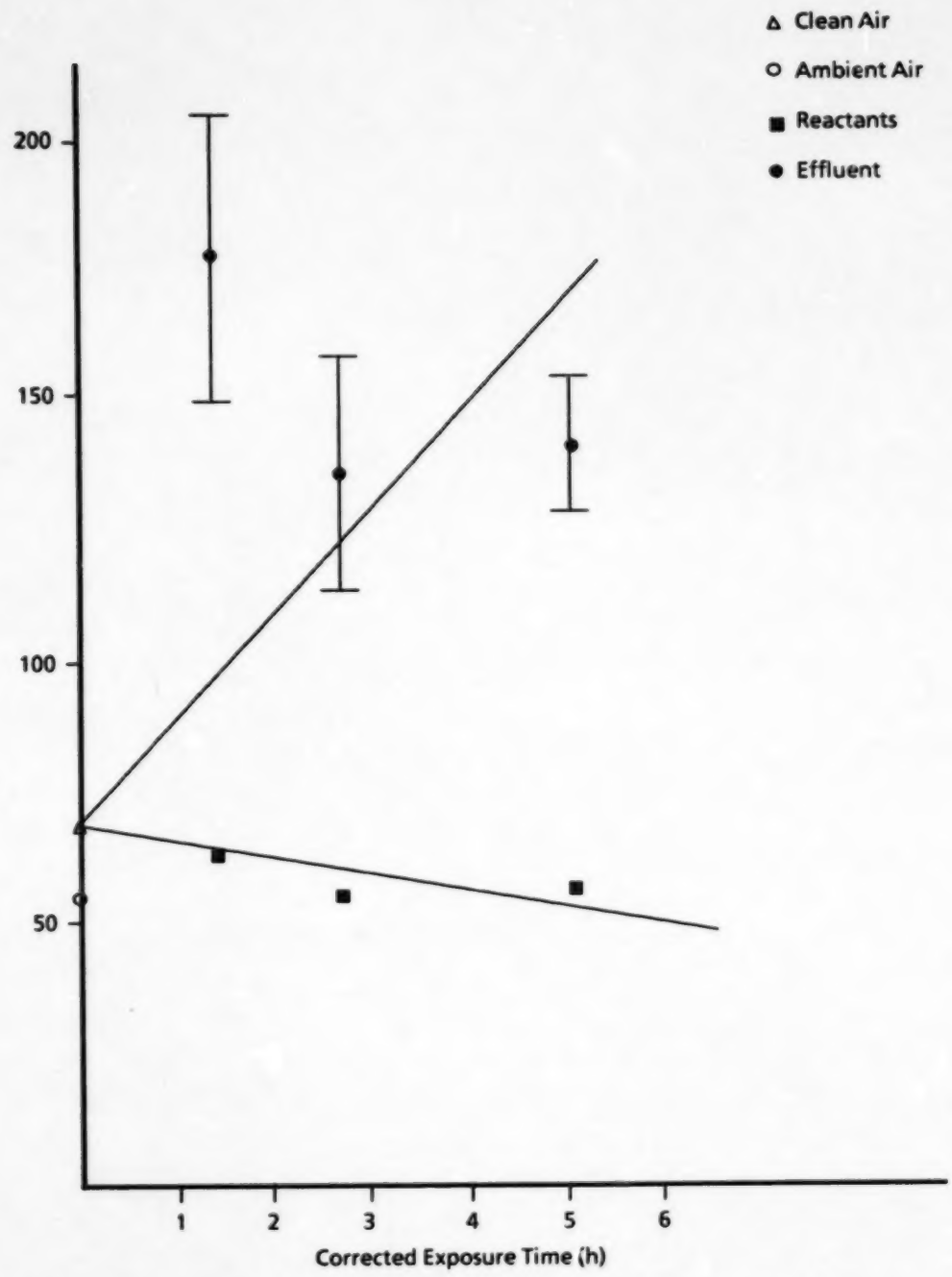

Figure C-4. Dose-response curve for the mutagenic activity (TA98 + S9) of the gas-phase components of wood smoke (+ 500 ppb NO_X) before and following irradiation.

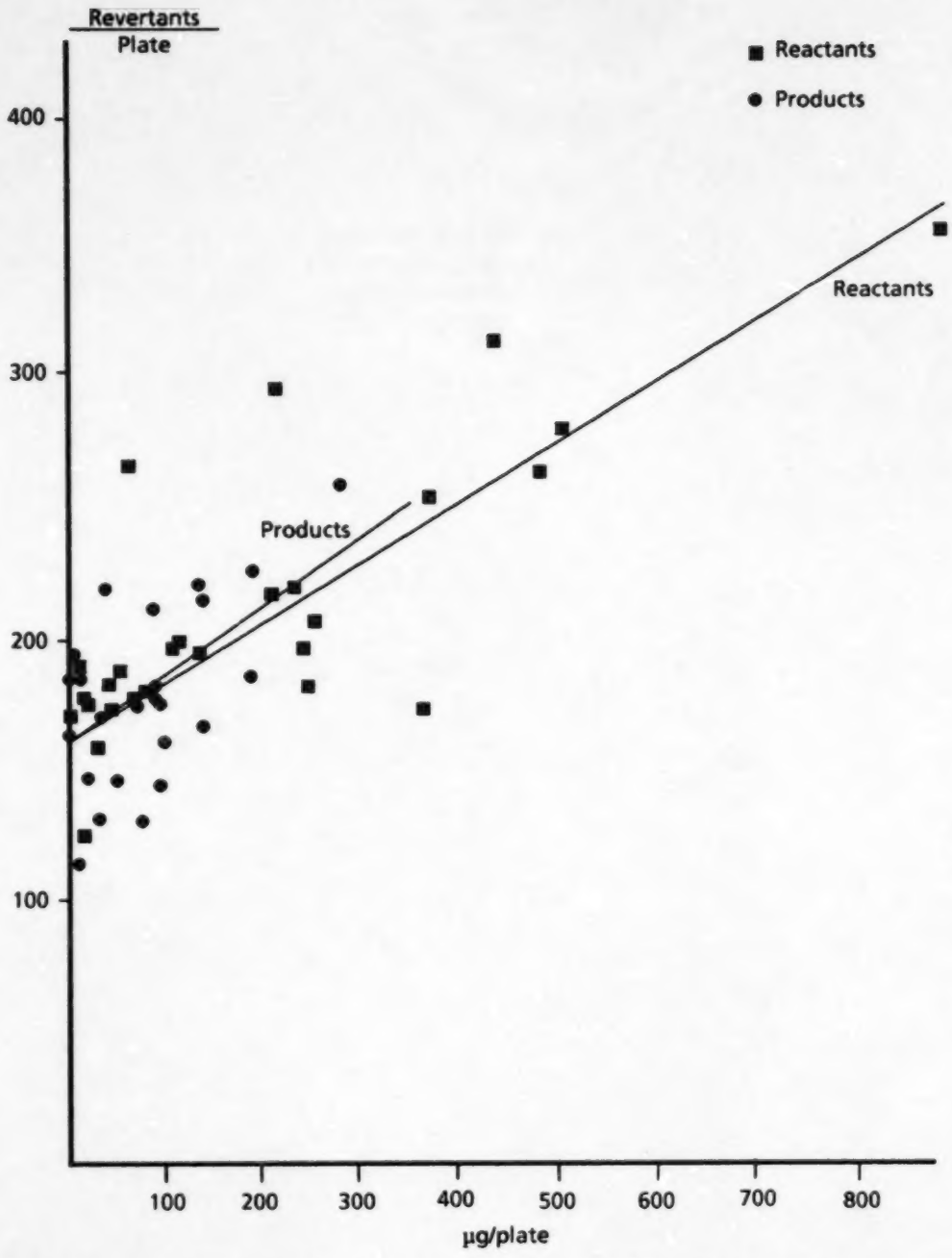

Figure C-5. Dose-response curve for the mutagenic activity (TA100 + S9) of the particulate extracts of wood smoke (+ 500 ppb NO_X) before and following irradiation.

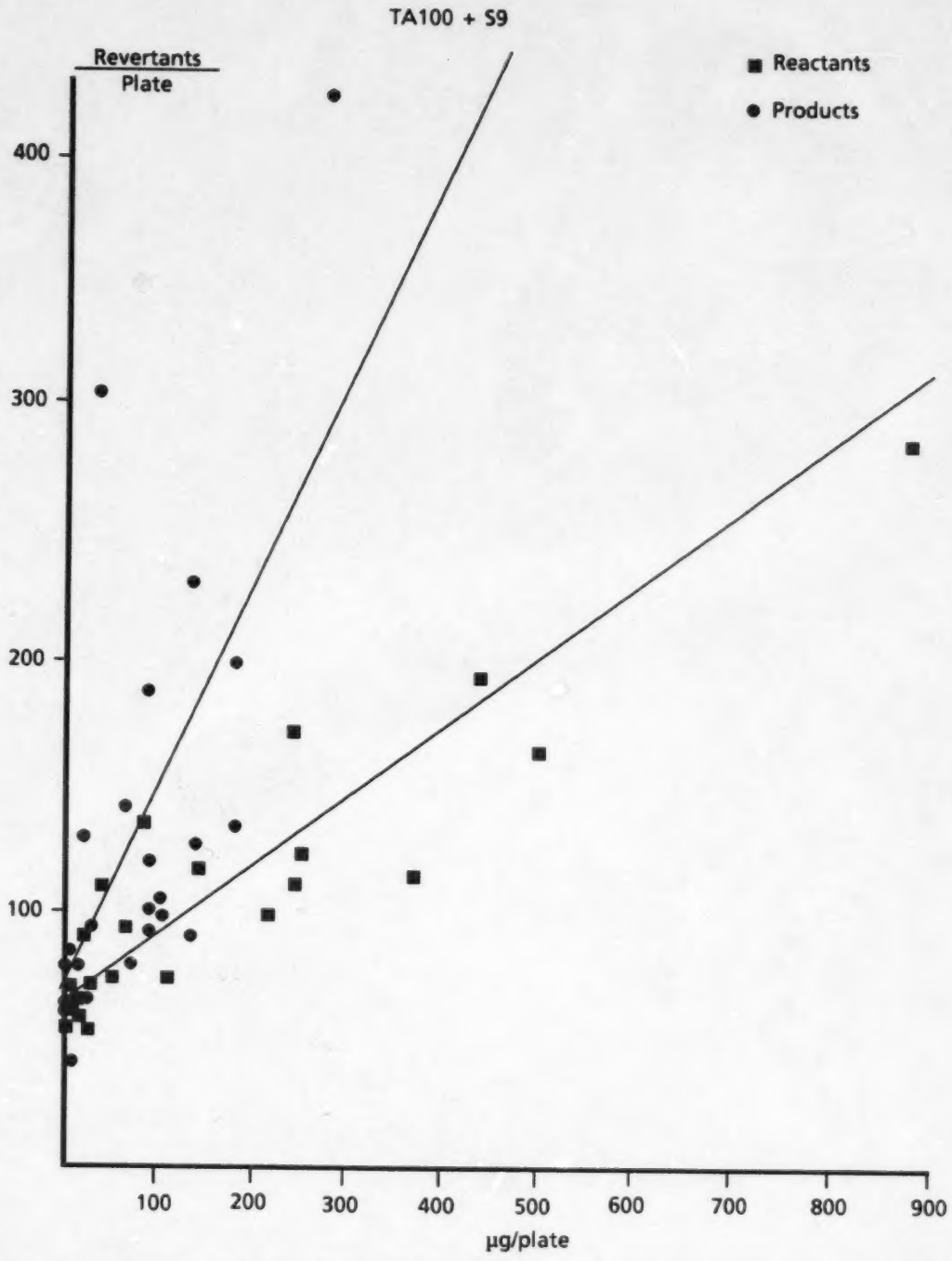

Figure C-6. Dose-response curve for the mutagenic activity (TA98 + S9) of the particulate extracts of wood smoke (+ 500 ppb NO_X) before and following irradiation.